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Assessment of Postischemic Neurogenesis in Rats with Cerebral Ischemia and Propofol Anesthesia

Irina Lasarzik, M.D., Uta Winkelheide, D.V.M.,* Sonja Stallmann, M.D.,* Christian Orth, M.D.,* Astrid Schneider, M.Sci.,‡ Achim Tresch, M.Sci.,§ Christian Werner, M.D., Ph.D.,‖ Kristin Engelhard, M.D., Ph.D.#*

Background: **Postischemic endogenous neurogenesis can be dose-dependently modulated by volatile anesthetics. The intravenous anesthetic propofol is used during operations with a risk of cerebral ischemia, such as neurosurgery, cardiac surgery, and vascular surgery. The effects of propofol on neurogenesis are unknown and, therefore, the object of this study.**

Methods: **Eighty male Sprague-Dawley rats were randomly assigned to treatment groups with propofol administration for 3 h: 36 mg · kg^{-1} · h^{-1} propofol with or without cerebral ischemia and 72 mg · kg^{-1} · h^{-1} propofol with or without cerebral ischemia. In addition, 7 rats with propofol administration for 6 h and 14 treatment-naive rats were investigated. Forebrain ischemia was induced by bilateral carotid artery occlusion and hemorrhagic hypotension. Animals received 5-bromo-2-deoxyuridine for 7 days. 5-Bromo-2-deoxyuridine–positive neurons were counted in the dentate gyrus after 9 and 28 days. Spatial learning in the Barnes maze and histopathologic damage of the hippocampus were analyzed.**

Results: **Propofol revealed no impact on basal neurogenesis. Cerebral ischemia increased the amount of new neurons. After 28 days, neurogenesis significantly increased in animals with low-dose propofol administered during cerebral ischemia compared with naive animals, whereas no significant difference was observed in animals with high-dose propofol during ischemia. Neuronal damage in the CA3 region was increased at 28 days with high-dose propofol. Postischemic deficits in spatial learning were not affected by propofol.**

Conclusions: **Independent effects of propofol are difficult to ascertain. Peri-ischemic propofol administration may exert secondary effects on neurogenesis by modulating the severity of histopathologic injury and thereby regenerative capacity of the hippocampus.**

IN the past, acute neurodegeneration was believed to result in permanent loss of neurons, with no capacity for cellular regeneration. This view has been challenged by the evidence that certain brain areas retain the capability to generate new neurons in adulthood.[1] The hippocampus is one of the major adult neurogenic regions.[1,2] Intrinsic stem cells in the adult hippocampus generate neurons that integrate into the existing neuronal network. Stem cells give rise to other stem cells as well as progenitor cells. Progenitor cells divide into neurons, astrocytes, and oligodendrocytes.[3]

Natural replacement of neurons and synapses may contribute to functional recovery after cerebral ischemia.[4] Newly generated neurons in the adult dentate gyrus make functional synaptic connections with the hippocampal circuitry and possess neurophysiologic characteristics of mature neurons.[5,6] Likewise, hippocampal neurogenesis supports spatial learning and memory.[7–9]

The existence of neurogenesis potentially offers a pharmacologic target for purposes of brain repair. Recent experiments in rats show that the amount of newly generated neurons in the hippocampus is dose-dependently affected by sevoflurane 28 days after forebrain ischemia.[10] The intravenous anesthetic propofol is popular for operations with a risk of cerebral ischemia, such as neurosurgery, cardiac surgery, and vascular surgery. It decreases intracranial pressure, maintains cerebrovascular autoregulation, and possesses a short context-sensitive half-life, which enables rapid recovery from anesthesia to control for surgical complications. However, the effects of propofol on endogenous neurogenesis after cerebral lesions are unknown. Therefore, the current study investigates the effects of propofol on adult neurogenesis in a rat model of forebrain ischemia.

Materials and Methods

Animals

All experiments were approved by the local governmental authorities (Landesuntersuchungsamt Rheinland-Pfalz, Koblenz, Germany; approval number 1.5 177-07/051-13) and performed in accordance with German animal protection law. One hundred one male Sprague-Dawley rats (354 ± 43 g; Charles River Laboratories, Sulzfeld, Germany) were included in three study sections (table 1). Proliferation of endogenous stem cells was assessed 9 days after cerebral ischemia (Isch09). Differentiation of newly generated neurons was analyzed 28 days after cerebral ischemia (Isch28). The effect of long-term propofol administration (6 h) on neurogenesis was assessed after 9 days (Prop6h). Nonanesthetized and nonischemic naive animals were studied after 9 and 28 days as natural reference.

* Resident, ‖ Professor of Anesthesiology and Chairman, # Associate Professor of Anesthesiology, Klinik für Anästhesiologie, ‡ Research Associate, § Assistant Professor, Institut für Medizinische Biometrie, Epidemiologie und Informatik, Johannes Gutenberg-Universität.

Received from Klinik für Anästhesiologie, Johannes Gutenberg-Universität, Mainz, Germany. Submitted for publication January 11, 2008. Accepted for publication October 17, 2008. Support was provided solely from institutional and/or departmental sources. Presented in part at the Annual Meeting of the American Society of Anesthesiologists and the Society of Neurosurgical Anesthesia and Critical Care, Chicago, Illinois, October 13–14, 2006, and the Annual Meeting of the American Society of Anesthesiologists and the Society of Neurosurgical Anesthesia and Critical Care, San Francisco, California, October 12–15, 2007. Data in this study form part of doctoral theses presented by Sonja Stallmann, M.D. (Resident, Klinik für Anästhesiologie, Johannes Gutenberg-Universität, Mainz, Germany), and Christian Orth (Resident, Klinik für Anästhesiologie, Johannes Gutenberg-Universität, Mainz, Germany) to the Medical Faculty, Johannes Gutenberg-Universität, Mainz, Germany.

Address correspondence to Dr. Lasarzik: Klinik für Anästhesiologie, Johannes Gutenberg-Universität, Langenbeckstr. 1, 55131 Mainz, Germany. lasarzik@uni-mainz.de. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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Table 1. Treatment Groups

Study	Group	Days of Surveillance	Treatment	n
Isch09	Prop36sham	9	36 mg · kg^{-1} · h^{-1} propofol, sham operation	10
	Prop36isch	9	36 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia	10
	Prop72sham	9	72 mg · kg^{-1} · h^{-1} propofol, sham operation	10
	Prop72isch	9	72 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia	10
	Naive	9	No anesthesia, no cerebral ischemia	7
Isch28	Prop36sham	28	36 mg · kg^{-1} · h^{-1} propofol, sham operation	10
	Prop36isch	28	36 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia	10
	Prop72sham	28	72 mg · kg^{-1} · h^{-1} propofol, sham operation	10
	Prop72isch	28	72 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia	10
	Naive	28	No anesthesia, no cerebral ischemia	7
Prop6h		9	6 h propofol (90–30 mg · kg^{-1} · h^{-1}), no cerebral ischemia	7

Isch09 = study assessing proliferation of endogenous stem cells 9 days after cerebral ischemia; Isch28 = study analyzing differentiation of newly generated neurons 28 days after cerebral ischemia; Prop6h = study assessing the effect of long-term propofol administration (6 h) on neurogenesis after 9 days.

Preparation and Cerebral Ischemia

Fasted rats were anesthetized in a bell jar saturated with sevoflurane (Sevorane®; Abbott GmbH, Wiesbaden, Germany), intubated, and mechanically ventilated (683 Small Animal Ventilator; Harvard Apparatus, Holliston, MA) with 3.5% sevoflurane in oxygen and air (fraction of inspired oxygen = 0.33). A temperature probe was placed into the right temporal muscle for monitoring and maintenance of pericranial temperature at 37°C with a servocontrolled heating lamp and pad (TCAT-2DF Controller; Physitemp Instruments Inc., Clifton, NJ). Catheters were inserted into the right femoral artery and vein and the right jugular vein for arterial blood pressure monitoring, blood withdrawal, and drug administration. Loose ligatures were placed around both common carotid arteries for later clamping. Incisions were infiltrated with bupivacaine (Carbostesin®; AstraZeneca GmbH, Wedel, Germany), sevoflurane was discontinued, and animals were randomly assigned to propofol treatment groups (table 1). In addition, animals received a sufentanil infusion at the rate of 2.5 μg · kg^{-1} · h^{-1} (Sufenta mite®; Janssen-Cilag GmbH, Neuss, Germany).

After an equilibration period of 30 min, forebrain ischemia was induced by blood withdrawal to a mean arterial blood pressure (MAP) of 40 mmHg and bilateral clip occlusion of the common carotid arteries. After 14 min of ischemia, clips were removed, and the withdrawn blood was reinfused slowly over 15 min for avoidance of cerebral hyperemia and systemic hypertension. Animals were kept in the same anesthesia protocol for a recovery period of 90 min (table 2). Sham-operated rats were subjected to the same operative procedures but did not undergo ischemia. Physiologic variables were recorded before cerebral ischemia (baseline), at the end of ischemia (ischemia), after reperfusion (reperfusion), and at the end of the recovery period (recovery). For sham-operated animals, variables were recorded at corresponding times. After completion of the recovery period, catheters were removed, wounds were closed, and animals were extubated. Pericranial temperature was monitored until the animals showed adequate motor activity. Then rats were returned to their cages.

Propofol Infusion

Propofol (Disoprivan®; AstraZeneca GmbH) was continuously administered for 3 h (Syringe Pump, Model 11 Plus; Harvard Apparatus). The rate of infusion was chosen according to the randomization with a low-dose rate of 36 mg · kg^{-1} · h^{-1} (= effective dose 50)[11] and a high-dose rate of 72 mg · kg^{-1} · h^{-1}. High-dose propofol groups received norepinephrine (Arterenol®; Aventis Pharma Deutschland GmbH, Frankfurt am Main, Germany) for avoidance of arterial hypotension according to requirements (dose range 0.01–0.14 μg · kg^{-1} · min^{-1}).

An additional group was used to assess effects of long-term (6 h) high-dose propofol infusion (Prop6h) on natural neurogenesis according to the following protocol: 90 mg · kg^{-1} · h^{-1} for 20 min, 80 mg · kg^{-1} · h^{-1} up to 1 h, 60 mg · kg^{-1} · h^{-1} up to 2 h, 40 mg · kg^{-1} · h^{-1} up to 4 h, 35 mg · kg^{-1} · h^{-1} up to 5 h, and 30 mg · kg^{-1} · h^{-1}

Table 2. Time Intervals

Treatment	Time, min	Anesthesia
Preparation	1–30	3–3.5 vol% sevoflurane
Equilibration	31–60	Discontinuation of sevoflurane, propofol infusion according to randomization
Blood withdrawal, cerebral ischemia and reperfusion	61–97	Propofol infusion according to randomization
Recovery	98–187	Propofol infusion according to randomization
Wound closure	188–200	Propofol infusion according to randomization

Time intervals in sequence for each treatment procedure. For sham-operated animals, cerebral ischemia was not performed, but time intervals were chosen accordingly.

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up to 6 h. This anesthesia protocol induces an electroencephalographic burst suppression ratio of 1–5% in rats.[12] MAP was adjusted with norepinephrine (dose range 0.02–0.28 $\mu g \cdot kg^{-1} \cdot min^{-1}$).

5-Bromo-2-deoxyuridine Labeling and Tissue Preparation

For labeling of proliferating cells, 100 mg/kg 5-bromo-2-deoxyuridine (BrdU; Sigma-Aldrich Chemie GmbH, Munich, Germany) was injected intraperitoneally every day during the first 7 days of survival. As a thymidine analog, BrdU is incorporated into DNA on mitotic division. After an observation period of 9 or 28 days, animals were deeply anesthetized with sevoflurane and transcardially perfused with 100 ml saline, 0.9%, and 100 ml paraformaldehyde, 4%, in phosphate buffer (0.2 M). Brains were removed, postfixed in paraformaldehyde–phosphate buffer for 24 h, and placed in 30% sucrose. Forty-micrometer sagittal brain sections were prepared and stored in a cryoprotection solution (glycerol, ethylene glycerol, and 0.1 M phosphate buffer) at −20°C.

Evaluation of Neurogenesis

Immunohistochemistry and immunofluorescence stainings were performed as previously described.[10] In brief, sections for immunohistochemistry staining were incubated with the primary mouse anti-BrdU antibody (monoclonal mouse immunoglobulin G [IgG], 1:500; Roche Diagnostics Corp., Indianapolis, IN) followed by an incubation with the biotinylated secondary donkey anti-mouse antibody (Biotin-SP–conjugated donkey anti-mouse IgG, 1:500; Jackson ImmunoResearch Laboratories Inc., West Grove, PA).

Sections for immunofluorescence staining were incubated with the primary rat anti-BrdU antibody (monoclonal rat IgG, 1:500; Oxford Biotechnology, Oxford, United Kingdom) and the primary goat anti-doublecortin antibody (polyclonal goat IgG, 1:500; Santa Cruz Biotechnology, Santa Cruz, CA; 9 days of survival) or the primary mouse anti-neuron–specific nuclear protein antibody (monoclonal mouse IgG, 1:250; Chemicon International, Temecula, CA; 28 days of survival) followed by incubation with the secondary antibodies (fluorescein isothiocyanate–conjugated donkey anti-rat IgG, 1:500, Rhodamine Red-X–conjugated donkey anti-goat IgG, 1:500, for 9 days of survival, or Rhodamine Red-X–conjugated donkey anti-mouse IgG, 1:500, for 28 days of survival; Jackson ImmunoResearch Laboratories Inc.).

Blinded stereologic analysis of immunohistochemically stained BrdU-positive cells was performed manually using light microscopy assuming equal distribution of BrdU-positive cells throughout the dentate gyrus. BrdU-positive cells were counted in a 1-in-10 series of sections (400 μm apart) spanning the entire dentate gyrus, and results were multiplied by 10. To determine the percentage of newly generated neurons, BrdU-positive cells were analyzed for colabeling of BrdU and doublecortin or neuron-specific nuclear protein with an immunofluorescence microscope combined with an ApoTome and Axiovision software (Axiovert 200; Zeiss GmbH, Göttingen, Germany). The resulting percentages were multiplied with the stereologically estimated amount of BrdU-positive cells assessed by immunohistochemical staining to estimate the amount of newborn neurons in the dentate gyrus.[13]

Evaluation of Histopathologic Damage and Volume of Dentate Gyrus

Histopathologic damage was assessed in the hippocampal CA1 and CA3 regions in sections stained with hematoxylin and eosin in a blinded fashion. The amount of injured neurons (eosinophilic cytoplasm and pyknotic nuclei) was graded according to the following score (hematoxylin–eosin index): 0 = no pathologic change, 1 = 1–10% pathologic change, 2 = 11–50% pathologic change, 3 = greater than 50% pathologic change.

The area of the dentate gyrus was measured in every 10th section of the brain using Optimas 6.51 (Media Cybernetics Inc., Silver Spring, MD). The volume of the dentate gyrus was calculated by multiplying the results with the thickness of the slice (40 μm) by 10 (because every 10th slice was analyzed).

Cognitive Outcome

To assess hippocampus-dependent place recognition, a modified Barnes maze was used.[14] The maze was a 160 × 160-cm platform mounted 80 cm above the floor, with eight equally spaced holes in a circle with a radius of 70 cm. Two large posters were mounted on the walls closest to the maze as spatial clues. The goal box was 20 × 15 × 12 cm and located in the same spatial position for all trials. Because learning a specific type of task can increase neurogenesis,[15] behavioral testing was performed solely in the 28-day survival group. Rats received two trials on postoperative day 22, and one trial each on postoperative days 24, 26, and 28. Rats were accustomed to the goal box for 1 min before each trial. Each trial was started by placing the animal in a start box in the middle of the maze and removing the start box to begin the trial. Rats were tested using bright light and white noise as aversive stimuli. Latency to goal box was recorded when all four legs were in the goal box. The time limit was 5 min for each trial. After each trial, the maze was cleaned with 70% ethanol. To avoid bias, the investigator was blinded to the treatment.

Statistical Analyses

The numbers of newly generated neurons are presented as median and quartiles, and statistical analyses were performed with a Mann–Whitney test. The significance level was defined at 5%, and Bonferroni adjustment was performed to account for multiple testing.

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Table 3. Physiologic Variables for Study Isch09

		Time			
	Group	Baseline	Ischemia	Reperfusion	Recovery
MAP, mmHg	Prop36sham	107 ± 23	103 ± 23	105 ± 20	96 ± 13
	Prop36isch	115 ± 21	40 ± 1*	130 ± 17	90 ± 18
	Prop72sham	93 ± 22	78 ± 13	77 ± 16	73 ± 9
	Prop72isch	87 ± 18	40 ± 1*	85 ± 12	69 ± 16
Pao_2, mmHg	Prop36sham	135 ± 12	132 ± 21	141 ± 27	132 ± 21
	Prop36isch	132 ± 21	166 ± 26	142 ± 18	121 ± 24
	Prop72sham	138 ± 29	128 ± 24	126 ± 25	108 ± 32
	Prop72isch	126 ± 17	120 ± 16	118 ± 14	105 ± 18
$Paco_2$, mmHg	Prop36sham	39 ± 3	40 ± 4	40 ± 5	40 ± 3
	Prop36isch	40 ± 4	38 ± 5	42 ± 2	41 ± 3
	Prop72sham	40 ± 4	41 ± 4	42 ± 3	41 ± 3
	Prop72isch	41 ± 3	42 ± 5	46 ± 5	40 ± 3
Hb, g/dl	Prop36sham	12.7 ± 1.4	12.7 ± 1.0	12.9 ± 1.1	13.3 ± 0.8
	Prop36isch	12.9 ± 1.0	11.3 ± 0.8	12.7 ± 0.8	12.7 ± 0.5
	Prop72sham	12.8 ± 1.9	12.3 ± 1.6	12.6 ± 1.2	12.6 ± 0.8
	Prop72isch	13.2 ± 0.9	11.4 ± 0.8	12.5 ± 0.9	12.2 ± 0.7
Gluc, mg/dl	Prop36sham	73 ± 22	61 ± 15	62 ± 13	74 ± 11
	Prop36isch	83 ± 16	121 ± 37	63 ± 10	64 ± 13
	Prop72sham	82 ± 15	61 ± 16	66 ± 17	70 ± 10
	Prop72isch	79 ± 14	100 ± 41	52 ± 12	53 ± 11

Mean arterial blood pressure (MAP), arterial oxygen tension (Pao_2), arterial carbon dioxide tension ($Paco_2$), hemoglobin concentration (Hb), and plasma glucose concentration (Gluc) during baseline (22 min before the onset of reperfusion) at the end of ischemia (1 min before the onset of reperfusion), reperfusion (15 min after the onset of reperfusion), and recovery (105 min after the onset of reperfusion) for animals surviving 9 days (Isch09). Data are presented as mean ± SD.

* Controlled parameter.

Prop36isch = 36 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia; Prop36sham = 36 mg · kg^{-1} · h^{-1} propofol, sham operation; Prop72isch = 72 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia; Prop72sham = 72 mg · kg^{-1} · h^{-1} propofol, sham operation.

Histopathologic damage was analyzed using Kruskal–Wallis tests and Mann–Whitney tests. Cognitive outcome in the Barnes maze was analyzed by a three-way analysis of variance including the variables group, treatment, and trial number. Statistical calculations were performed in SPSS 12.0 (SPSS, Inc., Chicago, IL). Physiologic parameters are presented as mean ± SD. No statistical tests were performed for physiologic parameters to avoid type I error inflation due to their large number. However, comparability of physiologic parameters across experimental groups was assessed on a descriptive level.

Results

Physiologic Parameters

Physiologic variables are listed in tables 3–5. MAP decreased in the ischemic groups to 40 mmHg by blood withdrawal. Average MAP of the pooled ischemic groups varied between low-dose and high-dose propofol (125 ± 18 *vs.* 83 ± 12 mmHg during reperfusion and 88 ± 15 *vs.* 71 ± 18 mmHg during recovery). Mean arterial oxygen tension ranged between 105 and 166 mmHg, with a maximum SD of 36 mmHg for all groups. Mean arterial carbon dioxide tension ranged between 38 and 46 mmHg, with a maximum SD of 10 mmHg for all groups. Because of blood withdrawal, hemoglobin concentration decreased by 1.8 g/dl on average at the end of ischemia. This was reversed after reperfusion. Plasma glucose concentrations of all ischemic groups increased during ischemia (from 78 ± 12 to 110 ± 35 mg/dl on average) but recovered after reperfusion (65 ± 13 mg/dl on average). With the exception of lower plasma glucose levels for group Prop72isch in study Isch09 (52 ± 12 mg/dl at reperfusion and 53 ± 11 mg/dl at recovery), values remained within the physiologic range of rats.

Neurogenesis

The number of newly generated neurons in the dentate gyrus of both hemispheres after 9 days of survival is shown in figure 1A. Propofol administration for 3 h did not affect neurogenesis in sham-operated rats (Prop36sham median 5,000 [Q1: 4,400; Q3: 6,700] new neurons, Prop72sham median 4,200 [Q1: 3,700; Q3: 5,600] new neurons) compared with naive rats (median 7,600 [Q1: 4,800; Q3: 8,500] new neurons). Nine days after cerebral ischemia, BrdU-positive neurons increased to a median of 16,300 [Q1: 12,700; Q3: 19,800] new neurons in group Prop36isch ($P < 0.05$ *vs.* Prop36sham and naive) and to a median of 14,600 [Q1: 13,300; Q3: 19,500] new neurons in group Prop72isch ($P < 0.05$ *vs.* Prop72sham and naive).

Figure 1B shows the amount of newly generated neurons in the dentate gyrus of both hemispheres after 28 days of survival. Propofol administration for 3 h did not alter the rate of neurogenesis in sham-operated rats (Prop36sham median 5,400 [Q1: 3,500; Q3: 8,500] new neurons, Prop72sham median 3,800 [Q1: 2,800; Q3:

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Table 4. Physiologic Variables for Study Isch28

		Time			
	Group	Baseline	Ischemia	Reperfusion	Recovery
MAP, mmHg	Prop36sham	94 ± 23	91 ± 24	90 ± 21	91 ± 23
	Prop36isch	92 ± 11	40 ± 1*	119 ± 19	86 ± 11
	Prop72sham	91 ± 17	79 ± 14	74 ± 12	74 ± 26
	Prop72isch	79 ± 10	40 ± 1*	81 ± 11	73 ± 20
Pao_2, mmHg	Prop36sham	129 ± 12	123 ± 16	127 ± 17	124 ± 18
	Prop36isch	124 ± 16	148 ± 26	138 ± 27	131 ± 22
	Prop72sham	133 ± 24	131 ± 27	134 ± 31	123 ± 36
	Prop72isch	138 ± 14	129 ± 19	135 ± 17	122 ± 15
$Paco_2$, mmHg	Prop36sham	40 ± 3	40 ± 5	40 ± 2	40 ± 3
	Prop36isch	41 ± 2	40 ± 6	43 ± 4	39 ± 3
	Prop72sham	39 ± 2	43 ± 10	42 ± 5	41 ± 2
	Prop72isch	40 ± 2	41 ± 5	42 ± 4	39 ± 2
Hb, g/dl	Prop36sham	12.6 ± 0.9	12.6 ± 0.5	12.6 ± 0.7	12.3 ± 0.6
	Prop36isch	12.7 ± 1.1	10.9 ± 0.7	12.6 ± 0.7	11.9 ± 1.1
	Prop72sham	13.0 ± 1.3	12.8 ± 0.9	13.1 ± 1.2	12.1 ± 0.9
	Prop72isch	13.2 ± 1.1	11.1 ± 0.8	12.3 ± 0.7	11.7 ± 1.2
Gluc, mg/dl	Prop36sham	84 ± 18	70 ± 9	72 ± 9	80 ± 10
	Prop36isch	66 ± 8	117 ± 40	72 ± 19	67 ± 11
	Prop72sham	90 ± 26	72 ± 9	77 ± 10	83 ± 16
	Prop72isch	85 ± 10	103 ± 20	67 ± 14	71 ± 13

Mean arterial blood pressure (MAP), arterial oxygen tension (Pao_2), arterial carbon dioxide tension ($Paco_2$), hemoglobin concentration (Hb), and plasma glucose concentration (Gluc) during baseline (22 min before the onset of reperfusion) at the end of ischemia (1 min before the onset of reperfusion), reperfusion (15 min after the onset of reperfusion), and recovery (105 min after the onset of reperfusion) for animals surviving 28 days (Isch28). Data are presented as mean ± SD.

* Controlled parameter.

Prop36isch = 36 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia; Prop36sham = 36 mg · kg^{-1} · h^{-1} propofol, sham operation; Prop72isch = 72 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia; Prop72sham = 72 mg · kg^{-1} · h^{-1} propofol, sham operation.

5,200] new neurons) compared with naive animals (median 6,000 [Q1: 3,800; Q3: 7,400] new neurons). Twenty-eight days after cerebral ischemia, the amount of BrdU-positive neurons increased to a median of 11,000 [Q1: 7,000; Q3: 17,200] new neurons in group Prop36isch ($P < 0.05$ *vs.* Prop36sham and naive) and to a median of 9,100 [Q1: 6,800; Q3: 10,700] new neurons in group Prop72isch ($P < 0.05$ *vs.* Prop72sham).

The amount of BrdU-positive neurons 9 days after extended high-dose propofol administration for 6 h is shown in figure 1C. Propofol administration for 6 h did not affect the number of newly generated neurons compared with the natural rate of neurogenesis of naive rats (median 7,600 [Q1: 4,400; Q3: 8,700] *vs.* median 6,300 [Q1: 5,700; Q3: 6,800] new neurons).

Table 5. Physiologic Variables for Study Prop6h

	Time			
	Baseline	1 h	3 h	5 h
MAP, mmHg	97 ± 11	84 ± 3	99 ± 12	96 ± 17
Pao_2, mmHg	168 ± 15	142 ± 12	142 ± 16	135 ± 15
$Paco_2$, mmHg	39 ± 2	44 ± 2	43 ± 3	42 ± 3
Hb, g/dl	13.8 ± 0.8	13.5 ± 0.8	12.9 ± 0.6	12.4 ± 0.6
Gluc, mg/dl	94 ± 10	77 ± 9	71 ± 9	75 ± 9

Mean arterial blood pressure (MAP), arterial oxygen tension (Pao_2), arterial carbon dioxide tension ($Paco_2$), hemoglobin concentration (Hb), and plasma glucose concentration (Gluc) during baseline and 1, 3, and 5 h after the beginning of high-dose propofol infusion for 6 h (Prop6h). Data are presented as mean ± SD.

Histopathologic Damage

Histopathologic damage of the hippocampus is displayed in figure 2. Sham-operated and naive animals showed no histopathologic damage. In ischemic rats, neuronal injury of the CA1 region was more than 50% with both propofol concentrations after 9 and 28 days. The CA3 region showed a minor damage (< 10%) regardless of the propofol concentrations 9 days after ischemia. After 28 days, injury of the CA3 region increased up to 50% with high-dose propofol, whereas it remained constant (< 10%) with low-dose propofol.

Volume of the Dentate Gyrus

The average volume of the dentate gyrus was similar for all groups (0.9 ± 0.1 mm^3), regardless of cerebral ischemia or propofol concentration (data not shown).

Cognitive Outcome

Figure 3 shows the results of the Barnes maze test. Cerebral ischemia reduced spatial learning ability after 4 weeks. Postischemic deficits in test performance were not affected by different propofol dosages.

Discussion

The current data show that propofol has no effect on neurogenesis in the dentate gyrus of nonischemic rats. Cerebral ischemia increased the amount of new neurons

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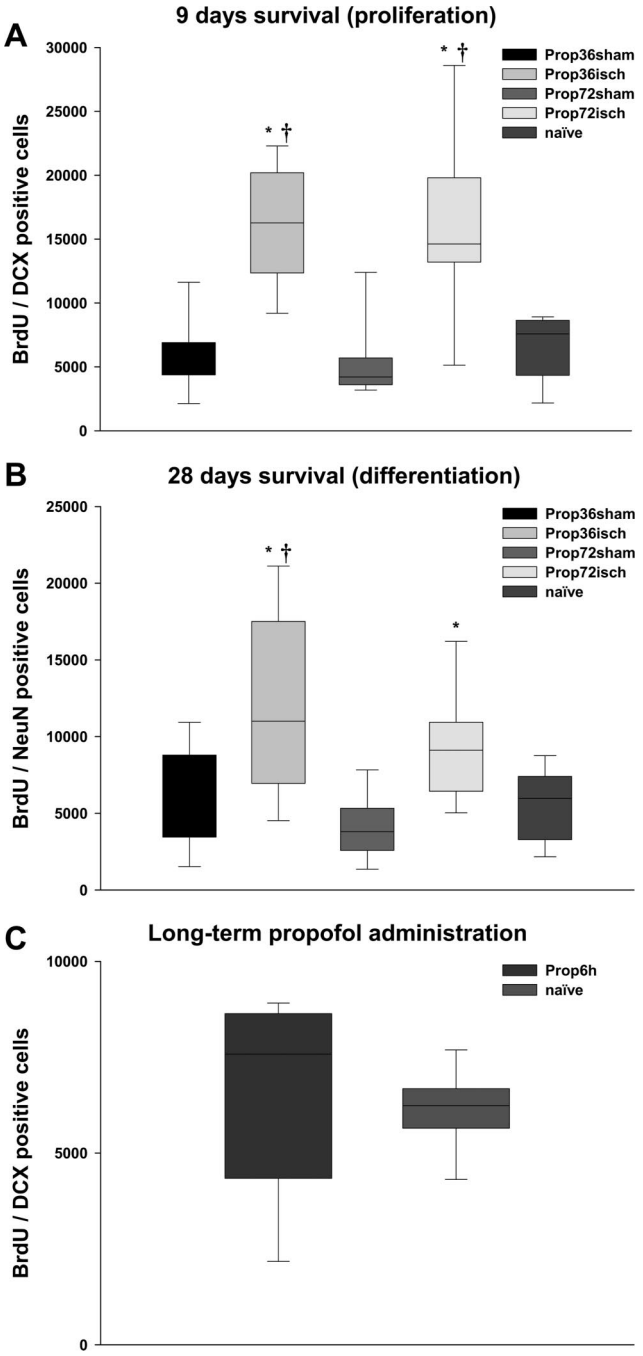

Fig. 1. Newly generated neurons in the dentate gyrus 9 days after cerebral ischemia (*A*), 28 days after cerebral ischemia (*B*), and 9 days after propofol anesthesia for 6 h (*C*). BrdU = 5-bromo-2-deoxyuridine; DCX = doublecortin; NeuN = neuron-specific nuclear protein; Prop6h = 6 h propofol (90–30 mg · kg^{-1} · h^{-1}), no cerebral ischemia; Prop36isch = 36 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia; Prop36sham = 36 mg · kg^{-1} · h^{-1} propofol, sham operation; Prop72isch = 72 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia; Prop72sham = 72 mg · kg^{-1} · h^{-1} propofol, sham operation. * *P* < 0.05 *versus* sham-operated animals with equal propofol dosage. † *P* < 0.05 *versus* naive animals (Bonferroni adjusted).

after 9 days, with no regard to peri-ischemic propofol concentrations. Likewise, neurogenesis was increased 28 days after ischemia, with no significant difference in the number of newly generated neurons when different propofol concentrations were compared directly. However, in animals with low-dose propofol postischemic neurogenesis increased significantly compared with naive rats, whereas no significant increase in differentiated neurons was observed with high-dose propofol administered during the ischemic episode.

The absent effect of propofol on neurogenesis in sham-operated animals agrees with a recent study where neurogenesis remained unaffected in sham-operated rats 28 days after 100 min of sevoflurane administration compared with the natural rate of neurogenesis in naive animals.[10] To exclude the possibility that the propofol administration was too short or the dosage was too low, a further group receiving high-dose propofol for 6 h (Prop6h) was performed. The applied propofol infusion protocol (90–30 mg · kg^{-1} · h^{-1}) resulted in deep anesthesia with an electroencephalographic burst suppression ratio of 1–5%.[12] However, the amount of newly generated neurons remained unaffected, indicating that propofol has no impact on the natural rate of neurogenesis.

Several challenges increase cell proliferation in the dentate gyrus, such as traumatic brain injury,[16] focal cerebral ischemia,[17] and global cerebral ischemia.[10] In rodents, the proliferation peak induced by ischemia is after 7–11 days.[18,19] Therefore, proliferation of neuronal stem cells was assessed 9 days after cerebral ischemia in the current study. The amount of new neurons increased significantly in both ischemic groups regardless of propofol concentrations after 9 days. This suggests that peri-ischemic propofol anesthesia has no dose-dependent impact on proliferation and short-term survival of neuronal stem cells after ischemic challenges.

The induction of neurogenesis after brain lesions indicates that this process is triggered by neuronal damage. In accord with this, a recent study in gerbils confirms that neurogenesis is dependent on neuronal death.[20] However, for long-term survival and differentiation of newly generated neurons, preservation of the CA3 region seems to be essential because new neurons need to establish axons projecting into the CA3 region.[21] Furthermore, synaptic contacts with the surrounding hippocampal circuitry are required for differentiation and survival of newly generated cells.[22] The current study therefore assessed the severity of hippocampal damage, showing that neuronal injury was significantly higher in the CA3 region with high-dose propofol compared with low-dose propofol after 28 days. The decrease in synaptic contacts due to severe histopathologic damage of the CA3 region may therefore have compromised the neuroregenerative potential of the hippocampus in the current study. Independent effects of propofol on differentiation and long-term survival of newly generated

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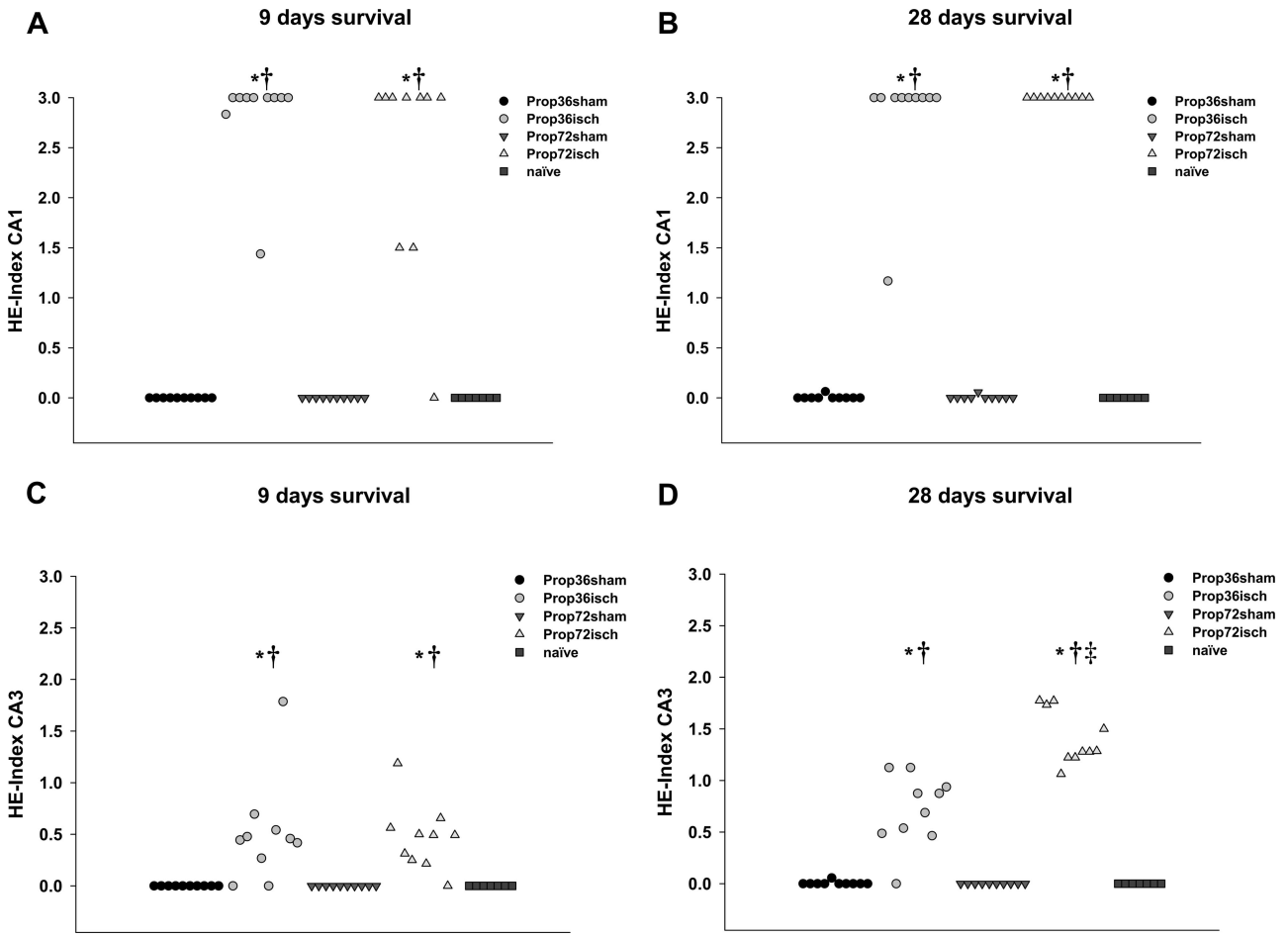

Fig. 2. Histopathologic damage of the CA1 region after 9 days (*A*) and after 28 days (*B*) and of the CA3 region after 9 days (*C*) and after 28 days (*D*). Neuronal damage is assessed as percentage of hippocampal neurons with pathologic changes (HE-Index = hematoxylin–eosin index): 0 = no pathologic change, 1 = 1–10% pathologic change, 2 = 11–50% pathologic change, 3 = greater than 50% pathologic change. Prop36isch = 36 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia; Prop36sham = 36 mg · kg^{-1} · h^{-1} propofol, sham operation; Prop72isch = 72 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia; Prop72sham = 72 mg · kg^{-1} · h^{-1} propofol, sham operation. * $P < 0.05$ *versus* sham-operated animals. † $P < 0.05$ *versus* naive animals. ‡ $P < 0.05$ *versus* Prop36isch.

neurons after 28 days are difficult to ascertain because propofol may modulate the extent of injury leading to a secondary but indirect effect on neurogenesis.

Because dose dependency is difficult to prove without a control for ischemia alone, an adequate control group to assess postischemic neurogenesis without interference of propofol would have been animals without anesthesia. However, this is not feasible in the bilateral common carotid artery occlusion model, because unanesthetized animals would not tolerate intravasal catheters needed for blood withdrawal and blood pressure control during performance of ischemia. Previous studies used fentanyl–nitrous oxide anesthesia for control groups in studies assessing the neuroprotective potential of anesthetic agents, because this ensures minimal effects on cerebral perfusion and metabolism.[23] Therefore, brains of animals anesthetized with fentanyl–nitrous oxide are comparable with brains of nonanesthetized animals. However, in the assessment of neurogenesis, high fentanyl doses may have presented a confounding factor, because μ-opioid receptors themselves take part in the regulation of progenitor cell survival, and a knockout of the μ-opioid receptor enhances the survival of adult-generated hippocampal neurons.[24] Models for focal cerebral ischemia, such as middle cerebral artery occlusion, yield the possibility to perform cerebral ischemia without interference of anesthetics.[25,26] However, an ischemia model with hypoperfusion of the frontal brain is clinically relevant for anesthesiology, because this may be caused by common anesthetic problems such as prolonged hypotension due to major blood loss or cardiac failure or during vascular and cardiac surgery.

Postischemic neuronal damage of the CA1 region was more than 50% regardless of propofol concentrations and survival period. The CA1 region is known as the most vulnerable region of the hippocampus, and global ischemia rapidly leads to a bilateral loss of CA1 pyramidal neurons.[27,28] Because the neuroprotective effects of anesthetic agents indirectly correlate with the severity of neuronal damage,[29] it is possible that in the current study the injury was too severe to allow for neuroprotection by propofol. In the less vulnerable CA3 region,

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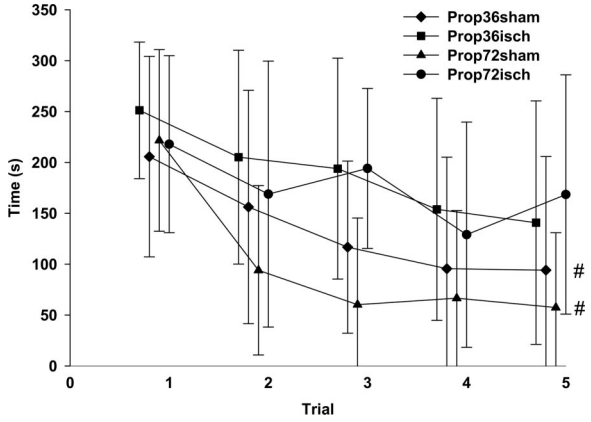

Fig. 3. Barnes maze trials. Spatial memory is assessed as time to the goal box in seconds. Prop36isch = 36 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia; Prop36sham = 36 mg · kg^{-1} · h^{-1} propofol, sham operation; Prop72isch = 72 mg · kg^{-1} · h^{-1} propofol, cerebral ischemia; Prop72sham = 72 mg · kg^{-1} · h^{-1} propofol, sham operation. # *P* < 0.05 *versus* ischemic rats.

minor damage was observed 9 days after cerebral ischemia independent of propofol concentrations. After 28 days, histopathologic damage progressed in animals with high-dose propofol, indicating that high-dose propofol was able to reduce necrotic cell death 9 days after cerebral ischemia, but did not reduce apoptotic cell death leading to neuronal death 28 days after injury. This is in contrast to previous studies showing sustained neuroprotection by propofol and might be attributed to different ischemic models (unilateral *vs.* bilateral carotid artery occlusion).[30] The possibility that delayed neuronal death is less effectively suppressed with high-dose than with low-dose propofol is in accord with experiments where increasing concentrations of anesthetics lead to a progressive loss of their neuroprotective potential.[31]

At 28 days from ischemia, spatial learning and memory was superior in sham-operated animals compared with rats with cerebral ischemia. Loss of hippocampal neurons after global ischemia produces a disorder of short-term memory and is associated with difficulty forming spatial memories.[32] Postischemic deficits in spatial memory were not affected by different propofol dosages. In the current study, the difference in the amount of newly generated neurons might be too small to account for an effect in spatial memory performance, because it has been demonstrated in contrast that cognitive recovery may reflect anatomical integration of newborn dentate granule neurons.[9]

In animals of the 28-day observation period, postischemic MAP values were considerably lower with high-dose propofol than with low-dose propofol. Because arterial hypotension deteriorates neurologic outcome after cerebral ischemia, this may present a confounding factor. However, postischemic MAP remained within a physiologic range where cerebral blood flow is independent of MAP due to cerebrovascular autoregulation, which is maintained up to 6 h after cerebral ischemia.[33] Furthermore, substantially greater differences in postischemic MAP are necessary to affect histologic outcome in this model, and the recovering brain demonstrates a surprising robustness to subsequent hypotensive challenges.[34] This may question a relation of postischemic MAP to the aggravation of histopathologic damage in the high-dose propofol group after 28 days. Accordingly, despite postischemic MAP differences between low- and high-dose propofol in animals of the 9-day observation period, histopathologic damage was comparable between these groups. Lower plasma glucose levels were observed for the high-dose propofol group with cerebral ischemia (9 days of survival) during reperfusion and recovery; however, this did not result in a difference in the severity of neuronal damage.

The use of sevoflurane as an induction agent may also present a methodologic issue in discerning the independent effect propofol on neurogenesis. Sevoflurane anesthesia in two different concentrations has demonstrated an increase in postischemic neurogenesis with higher sevoflurane concentrations in the same ischemia model as used in this study.[10] However, the 80% decrement time of sevoflurane is less than 8 min and does not increase significantly with the duration of anesthesia.[35] Therefore, it can be assumed that, following an equilibration period of 30 min after the end of sevoflurane administration, sevoflurane is almost completely eliminated, and an effect on postischemic neurogenesis is unlikely. Even if there was an overall effect of sevoflurane, it should not have influenced comparability of the groups, because the same dose of sevoflurane was given in all animals.

In conclusion, different concentrations of propofol did not affect hippocampal neurogenesis compared with natural neurogenesis, even when administration was extended to 6 h. Cerebral ischemia stimulated proliferation of new neurons 9 days after cerebral ischemia. This effect was sustained for at least 28 days. A significant dose-dependent effect of peri-ischemic propofol administration on postischemic endogenous neurogenesis could not be ascertained when ischemic groups were compared directly. However, in animals with low-dose propofol neurogenesis increased significantly compared with naive rats after 28 days, whereas no significant increase in differentiated neurons was observed with high-dose propofol administered during cerebral ischemia. This slight difference in the number of newly generated neurons may be attributed to a more severe damage of the hippocampal CA3 region in rats receiving high-dose propofol, because integrity of the CA3 region is important for effective neuroregeneration.

The authors thank Frida Kornes (Technician, Klinik für Anästhesiologie, Johannes Gutenberg-Universität, Mainz, Germany) for excellent technical assistance.

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