

Involvement of the Basal Cholinergic Forebrain in the Mediation of General (Propofol) Anesthesia

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Background: Recent studies have pointed out the involvement of the basal forebrain γ -aminobutyric acid–mediated system in mediating the effects of general anesthesia. In this study, the authors asked whether the basal forebrain cholinergic system is also involved in mediating the effects of general anesthetics such as propofol.

Methods: Cholinergic lesions were produced by administration of the selective immunotoxin 192 immunoglobulin G–saporin into the lateral ventricles, the medial septum, or the nucleus basalis magnocellularis. The anesthetic potency of propofol was determined using an anesthetic score with a crossover counterbalanced design. Animals were given intraperitoneal propofol (25 or 50 mg/kg) repeatedly every 15 min to set up a subanesthetic (low-dose) or anesthetic (high-dose) state. The anesthetic score was assessed for each cumulative dose. Control of the cholinergic depletion was performed using histochemical acetylcholinesterase staining on brain slices.

Results: A shift from a subanesthetic state to an anesthetic state was observed mainly in the rats with the immunotoxin injected into the lateral ventricles or the medial septum and vertical diagonal band of Broca, compared with controls. In those rats, the density of acetylcholinesterase reaction products was normal in the striatum and the thalamus, but reduced in the cortex and the hippocampus.

Conclusion: The anesthetic potency of propofol was increased in all rats with hippocampal lesions, whatever the injection sites, compared with controls. These results demonstrate that a cholinergic dysfunction in the basal forebrain potentiates the anesthetic effects of propofol.

RECENT studies have pointed out the involvement of specific parts of the forebrain in mediating the effects of general anesthesia^{1,2} and, more precisely, a septohippocampal influence on the effects of general anesthesia. The inactivation of the medial septum (MS) or the hippocampus by muscimol, a γ -aminobutyric acid type A

receptor agonist, decreased the dose of general anesthetic required to induce a loss of righting reflex in rats.² This participation of the γ -aminobutyric acid–mediated (GABAergic) septohippocampal system in mediating the effects of general anesthetics was evidenced for both volatile (halothane and isoflurane) and nonvolatile (propofol and pentobarbital) anesthetics. However, GABAergic targets represent only a subset of the cortically projecting and other forebrain neurons, which include also a large amount of central cholinergic neurons. To which extent the cholinergic basal forebrain also participates in mediating general anesthesia remains to be determined. This might be of clinical relevance because one morphofunctional change of the aged brain is an impairment of the basal forebrain cholinergic pathways.³

Central cholinergic neurotransmission has been recognized as playing a role in the modulation of general anesthesia.^{4–9} Drugs that affect the central cholinergic transmission have been shown to modulate the unconsciousness produced by anesthetics.^{10–14} For example, physostigmine, a reversible cholinesterase inhibitor that crosses the blood–brain barrier, increased the dose of propofol required to induce anesthesia¹³ and reversed the unconsciousness induced by propofol.¹⁴ Physiologic studies have largely evidenced the role of the basal forebrain cholinergic system in the maintenance of consciousness by modulating the shift between the desynchronized and synchronized states of cortical activation.¹⁵ The anesthetic state is also characterized by a shift in the electroencephalogram, from a desynchronized toward a synchronized electrical cortical activity, with parameters depending on the anesthetic agent used.^{16,17} The cholinergic activation of the cortex relies mainly on the thalamocortical pathway, but also on the cortical projections from the nucleus basalis magnocellularis (NBM), and the hippocampocortical pathways.^{18,19}

The purpose of the current approach was to further study the involvement of the different cholinergic forebrain pathways in mediating the effects of general anesthesia. To address this question, we performed selective cholinergic lesions in the basal forebrain in rats and determined the effects of incremental doses of the general anesthetic propofol on the setting of an anesthetic state. The cholinergic lesion in rats was performed using 192 immunoglobulin G (IgG)–saporin, a monoclonal antibody against the p75NGF receptor coupled to the ribosomal toxin saporin.^{20–25} This neurotoxin has been largely demonstrated to be highly selective for the cholinergic neurons of the basal forebrain.²⁶ First, we performed global cholinergic lesions of the basal fore-

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Received from Groupe de Recherche Exp erimentale sur les R percussions Cognitivo-affectives de l'anesth sie, Inserm U666 and Institut F d ratif des Neurosciences 37, Strasbourg, France. Submitted for publication June 24, 2007. Accepted for publication January 2, 2008. Supported by Institut National de la Sant  et de Recherche M dicale (INSERM), Paris, France; Minist re de l'Education Nationale de la Recherche et de la Technologie, Paris, France (projet de soutien des Instituts F d ratifs de Recherche aux sciences du vivant, Institut F d ratif de Recherche Neurosciences 37); and Universit  Louis Pasteur, Strasbourg, France (Appels   Projets exceptionnels). Dr. Laalou was supported by the Fondation pour la Recherche M dicale, Paris, France.

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brain using the injection of 192 IgG-saporin into the lateral ventricles (experiment 1) as previously described.^{20,22,27} When injected into the ventricles, this immunotoxin also reaches (and damages) part of the Purkinje cells in the cerebellum, where p75NGF receptors are found. Therefore, in two additional experiments, we examined the role of different structures of the cholinergic basal forebrain in mediating anesthesia by intraparenchymal administration of the immunotoxin. To this end, injections of 192 IgG-saporin were performed in either the NBM (experiment 2) or the MS/vertical limb of the diagonal band of Broca (vDBB) (experiment 3), as previously described.²⁸⁻³⁰ In that case, the toxin does not reach the cerebellum, and Purkinje cells are preserved.

Materials and Methods

This study, including care of the animals involved, was conducted according to the official edict presented by the French Ministry of Agriculture (Paris, France) and the recommendations of the Declaration of Helsinki. Therefore, these experiments were conducted in an authorized laboratory and under the supervision of an authorized researcher (J.-C.C., No. 6212, Animal Care Committee, Strasbourg, France). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Animals

Our subjects were 61 Long Evans male rats (Centre d'Élevage Rongeur Janvier, Le Genest St-Isle, France) weighing 200–220 g; they were 3 months old at the beginning of the experiments. All rats were housed in a colony room maintained on a 12:12 h dark-light cycle (lights on at 7:00 AM), with food and water provided *ad libitum*. The colony and testing rooms were under controlled temperature ($21^{\circ} \pm 2^{\circ}\text{C}$).

Drugs

Propofol (10 mg/ml, Diprivan; Astra-Zeneca, Paris, France) was prepared immediately before use and injected intraperitoneally in a volume of 2.5 ml/kg (25 mg/kg) or 5 ml/kg (50 mg/kg). The immunotoxin 192 IgG-saporin (Advanced Targeting Systems, San Diego, CA; stock solution: 2.3 $\mu\text{g}/\mu\text{l}$ phosphate-buffered saline; batch No. 17-11) was prepared immediately before use and injected in the lateral ventricles (experiment 1), in the nucleus basalis (experiment 2), or in the MS (experiment 3).

Surgery

During surgery, the rats were subjected to infusions of 192 IgG-saporin to induce a cholinergic lesion or vehicle as a control. All surgical procedures^{20,22} were conducted

under aseptic conditions using pentobarbital anesthesia (65 mg/kg, intraperitoneally; Sanofi, Libourne, France).

Experiment 1: Intracerebroventricular Injection. The rats were allocated to one of the two following groups: rats with saporin lesions (SAPO intracerebroventricular; $n = 14$) and sham-operated controls (SHAM intracerebroventricular; $n = 6$). After a mini craniotomy, injections into the lateral ventricles were performed stereotaxically through a 2- μl Hamilton syringe at the following coordinates: anterior: -0.8 mm (from bregma), lateral: ± 1.4 mm (from midline), ventral: -4.3 mm (from bregma), with the incisor bar set at the level of the interaural line.³¹ After the injection, the needle was left *in situ* for 5 min, retracted over 2 mm, and kept there for another delay of 4 min before complete retraction. Rats with saporin lesions (SAPO) received an intracerebroventricular injection of 2 μg of 192 IgG-saporin (2 μg per rat; 1 μl /lateral ventricle, concentration 1 $\mu\text{g}/\mu\text{l}$ phosphate-buffered saline). In the control group (SHAM), rats were operated in a similar manner, except that only phosphate-buffered saline was injected instead of 192 IgG-saporin.

Experiment 2: Nucleus Basalis Magnocellularis Injection. The rats were allocated to one of the two following groups: rats with saporin lesions (SAPO NBM; $n = 12$) and sham-operated controls (SHAM NBM; $n = 10$). After a mini craniotomy during anesthesia, the rats received stereotaxically guided injections of a total of 0.4 μg (in a total volume of 0.4 μl) 192 IgG-saporin into the NBM. The sham-lesioned rats received injections of phosphate-buffered saline. The coordinates according to the atlas of Paxinos and Watson³¹ were as follows: anterior: -1.0 mm (from bregma), lateral: ± 3.0 mm (from midline), ventral: -6.5 mm (from bregma), with the incisor bar set at the level of the interaural line. After each injection, the needle was left *in situ* for 6 min, retracted over 1 mm, and kept there for another delay of 4 min before complete retraction to prevent leakage of the toxin into the injection track.²⁸

Experiment 3: Medial Septum/Vertical Diagonal Band of Broca Injection. The rats were allocated to one of the two following groups: rats with saporin lesions (SAPO MS/vDBB; $n = 12$) and sham-operated controls (SHAM MS/vDBB; $n = 7$). After a mini craniotomy during anesthesia, the rats received stereotaxically guided injections of a total of 0.8 μg (in a total volume of 0.8 μl) 192 IgG-saporin into the MS/vDBB. The sham-lesioned rats received injections of phosphate-buffered saline. The coordinates according to the atlas of Paxinos and Watson³¹ were as follows: anterior: $+0.6$ mm (from bregma), lateral: ± 0.2 mm (from midline), ventral: -7.2 mm for the vDBB and ventral: -6.5 mm for the MS (from bregma), with the incisor bar set at the level of the interaural line. After each injection, the needle was left *in situ* for 6 min, retracted over 1 mm, and kept there for another delay of 4 min before complete retraction to prevent leakage of the toxin into the injection track.²⁷

Assessment of Anesthetic Scores

Four weeks after surgery, a delay largely sufficient for the lesions to achieve their maximal effect, the anesthetic potency of propofol was determined by setting up two different states (subanesthetic and anesthetic) using repeated injections of two different doses of propofol (25 or 50 mg/kg; interval 15 min) according to a cross-over counterbalanced design at a 2-week interval.

In each experiment, the effects of propofol were assessed as follows: the rat was placed in a cage in which the temperature was maintained at 28°C using an air-heating system. Propofol was repeatedly injected every 15 min. Ten minutes after the injection, each animal was tested for its "anesthetic score" by an experimenter who used the scale described hereafter. The experimenter was blind to the surgical treatment of the rat and could not deduce it from the behavior of the animal.

- 0.0: Spontaneous locomotor activity during a 1-min period of observation.
- 0.2: No spontaneous locomotor activity during a 1-min period of observation.
- 0.4: No motor response of orientation when placed on a grid inclined (45°) with the head down during a 30-s period of observation. When placed in such a position, nontreated rats usually exhibit movement to place the head up within less than a few seconds (approximately 4–8 s).
- 0.6: No righting reflex when placed on the back during a 30-s period of observation. When placed in such a position, nontreated rats display movements to leave it within a few seconds (approximately 4–8 s).
- 0.8: No paw withdrawal reflex in response to paw clamp pressure. We assessed the reflexive withdrawal of the leg after a pinch of 300 g; the pinch of 300 g was applied (*via* Electronic Von Frey Unit EVF3; Bioseb, Vitrolles, France) to the interdigit region of hind paw during 30 s. If no movement of the leg was observed, the paw withdrawal reflex was considered to be absent.
- 1.0: No eye-blink reflex in response to gentle application of a cotton tip to the cornea.

Animals in each group (SAPO or SHAM) received either propofol by steps of 25 mg/kg to set up a "subanesthetic state" (no loss of righting reflex, *i.e.*, anesthetic score <0.6) or propofol by 50 mg/kg to set up an "anesthetic state" (at least loss of righting reflex, *i.e.*, anesthetic score \geq 0.6; *cf.* fig. 1) in a counterbalanced order at an interval of 2 weeks. The comparison of the anesthetic scores in rats with saporin lesions with those of sham-operated controls allowed for assessment of the effects of the cholinergic lesion on the propofol-induced anesthesia.

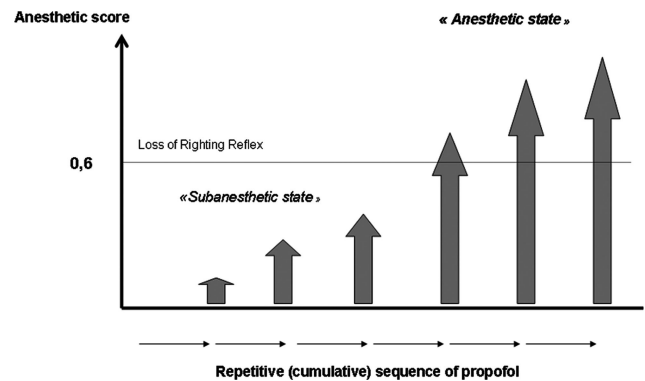


Fig. 1. Each animal received either intraperitoneal injection of propofol, every 15 min, by steps to set up a subanesthetic state (no loss of righting reflex, *i.e.*, anesthetic score <0.6) or an anesthetic state (at least loss of righting reflex, *i.e.*, anesthetic score \geq 0.6).

Histochemistry

Finally, for each animal, the extent of the cholinergic depletion was assessed by measuring the histochemical activity of acetylcholinesterase in the neocortex, the hippocampus, the thalamus, and the striatum on coronal brain sections.

To verify the efficiency of the cholinergic lesions, each rat was deeply anesthetized with pentobarbital (100 mg/kg, intraperitoneally) and subjected to a transcardiac perfusion of 50 ml saline followed by 50 ml paraformaldehyde (4%, 4°C) in phosphate-buffered saline. The brain was then removed, postfixed for 4 h in the same fixative, cryoprotected in 30% sucrose-saline (4°C) until it sank, and frozen at -70°C. Serial coronal sections (30 μ m thick) were cut using a cryostat microtome (-23°C) and were thaw mounted on gelatin-coated slides. Histochemical staining of acetylcholinesterase was used as a marker for cholinergic innervation using acetylthiocholine iodide (4 mM) as the substrate and ethopropazine (0.3 mM) as an inhibitor of nonspecific cholinesterases.²²

Observations of the sections stained for acetylcholinesterase activity were completed by quantification of optical density of acetylcholinesterase reactions products³⁰ in the frontal and parietal cortices, hippocampus, thalamus reticular nucleus, and striatum using a computer-assisted image analysis system (Biocom 500; Les Ulis, France). The density was measured bilaterally for each of these structures defined according to the rat brain atlas of Paxinos and Watson³¹ in a minimum of four brain sections. The mean optic density was considered as a "background" and subtracted from all measures before descriptive analysis was obtained from a value taken for each rat in the corpus callosum, where almost no acetylcholinesterase-positive reaction products could be identified.³⁰ The experimenter performing the optic density assessments was not aware of the rat's surgical treatment and rating scores.

Statistical Analyses

The statistical analysis was performed similarly for experiments 1, 2, and 3, using the software SYSTAT version 8.0 (Systat Software Inc., San Jose, CA).

For the behavioral experiments, the anesthetic score was considered as the dependent variable. A previous study found that the anesthetic score easily supports parametric analysis.³² Data were analyzed separately for each of the two propofol regimens: step of 25 mg/kg or step of 50 mg/kg, because there was no significant effect of the order of passage in each experiment (all $P < 0.05$). Statistical analyses were performed using two-way analysis of variance with SHAM or SAPO assignment as a between-subjects factor and cumulative dose as a repeated-measures factor.

For histochemical control, a multivariate analysis of variance was performed on the acetylcholinesterase optic density with SHAM or SAPO assignment or site of injection (intracerebroventricular, NBM, or MS/vDBB) assignment as between-subjects factors and the brain area as a repeated-measures factor. For each site of injection, one-way analyses of variance were performed on acetylcholinesterase optic density in the different brain structures (data from left and right hemispheres were averaged), namely the hippocampus, the frontal and parietal cortices, the striatum, and the thalamic reticular nucleus. An α level of 0.05 was considered indicative of statistical significance.

Results

Anesthetic Scores

Experiment 1: Intracerebroventricular Injection.

After the repetitive injection of 25 mg/kg (subanesthetic state), the anesthetic score increased as the cumulative dose of propofol was increased, with a more pronounced effect in lesioned animals as compared with the control ones (fig. 2A). At the cumulative dose of 175 mg/kg, we observed a shift from a subanesthetic state to an anesthetic state in SAPO rats but not in SHAM rats (anesthetic score ≥ 0.6). Analysis of variance for repeated measures showed a significant effect of the group factor ($F_{1,18} = 4.64$, $P < 0.05$), a significant effect of the cumulative dose of propofol ($F_{6,108} = 20.28$, $P < 0.05$), and a significant interaction between the two factors ($F_{6,108} = 2.62$, $P < 0.05$; fig. 2A).

After the repetitive injection of 50 mg/kg (anesthetic state), the anesthetic score increased as the cumulative dose of propofol was increased. This effect was more pronounced in lesioned animals as compared with the control ones (fig. 2B). Therefore, the anesthetic potency of propofol was significantly increased in SAPO rats as compared with SHAM rats. Analysis of variance for repeated measures showed a significant effect of the group factor ($F_{1,18} = 7.79$, $P < 0.05$) and of the cumulative dose of propofol ($F_{4,72} = 69.97$, $P < 0.05$; fig. 2B).

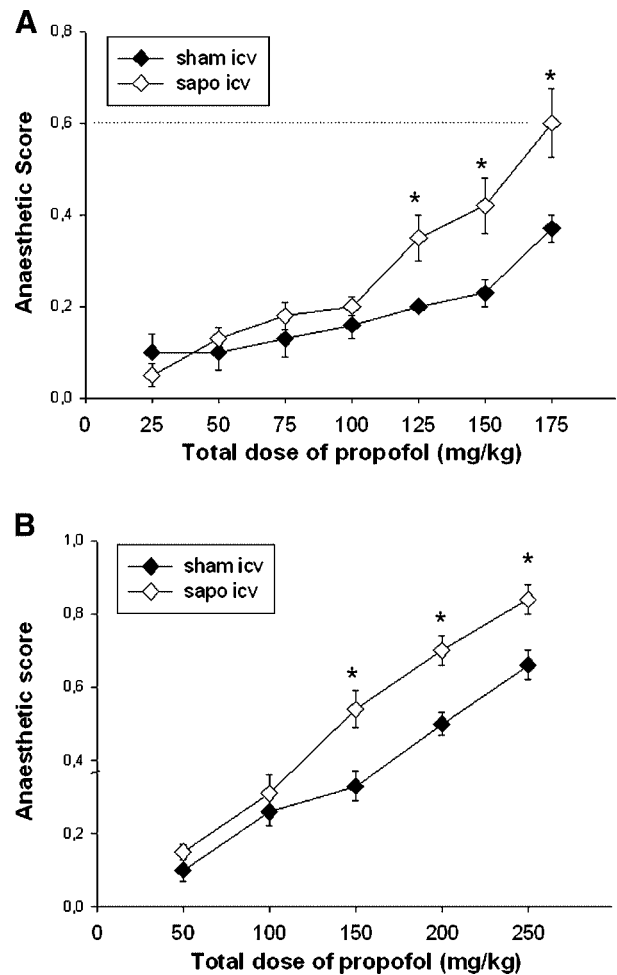


Fig. 2. The effects of propofol (intraperitoneally) were determined by the anesthetic score obtained for each cumulative dose in rats that sustained intraventricular injections (icv) of 192 immunoglobulin G-saporin (sapo; $n = 14$) or phosphate-buffered saline solution (sham; $n = 6$). Results are expressed as mean \pm SEM. (A) Subanesthetic state set by repeated administration of 25 mg/kg propofol. (B) Anesthetic state set by repeated administration of 50 mg/kg propofol. * $P < 0.05$ compared with SHAM-operated animals.

Experiment 2: Nucleus Basalis Magnocellularis Injection.

After the repetitive injection of 25 mg/kg (subanesthetic state), the anesthetic score increased as the cumulative dose of propofol was increased (fig. 3A). However, the anesthetic score did not differ in lesioned animals as compared with the control ones (fig. 3A). Analysis of variance for repeated measures showed no significant effect of the group factor, ($F_{1,20} = 0.09$) but there was a significant effect of the cumulative dose of propofol ($F_{6,120} = 36.07$, $P < 0.05$), with no interaction between the two factors.

After the repetitive injection of 50 mg/kg (anesthetic state), the anesthetic score increased as the cumulative dose of propofol was increased. This effect was more pronounced in lesioned animals as compared with the control ones (fig. 3B). Therefore, the anesthetic potency of propofol was significantly increased in SAPO rats as

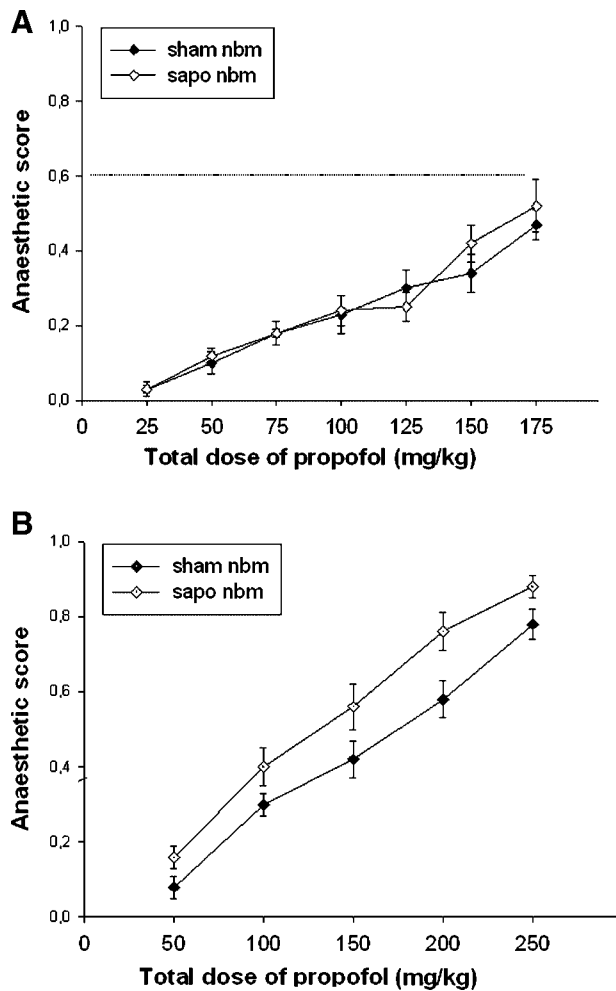


Fig. 3. The effects of propofol (intraperitoneally) were determined by the anaesthetic score obtained for each cumulative dose in rats that sustained injection of 192 immunoglobulin G-saporin (sapo; $n = 12$) or phosphate-buffered saline solution (sham; $n = 10$) into the nucleus basalis magnocellularis (nbm). Results are expressed as mean \pm SEM. (A) Subanesthetic state set by repeated administration of 25 mg/kg propofol. (B) Anesthetic state set by repeated administration of 50 mg/kg propofol.

compared with SHAM rats. Analysis of variance for repeated measures showed an overall significant effect of the group factor ($F_{1,18} = 5.50, P < 0.05$) and of the cumulative dose of propofol ($F_{4,72} = 93.34, P < 0.05$), but there was no significant interaction between the two factors ($F_{4,72} = 0.49$). It is particularly noteworthy that besides the overall group effect, there was no significant interaction. In fact, *post hoc* analyses using *t* tests with Bonferroni correction did not evidence any significant differences between lesioned animals as compared with the control ones, whatever the cumulative dose. Two lesioned rats died unexpectedly before being assessed.

Experiment 3: Medial Septum/Vertical Diagonal Band of Broca Injection. After the repetitive injection of 25 mg/kg (subanesthetic state), the anaesthetic score increased as the cumulative dose of propofol was in-

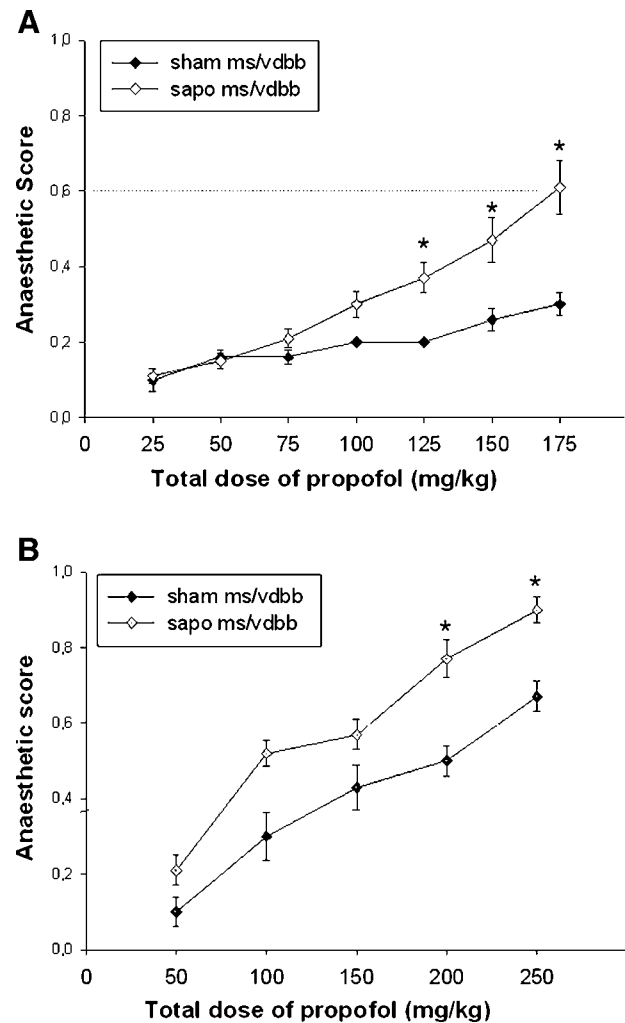


Fig. 4. The effects of propofol (intraperitoneally) were determined by the anaesthetic score obtained for each cumulative dose in rats that sustained injections of 192 immunoglobulin G-saporin (sapo; $n = 12$) or phosphate-buffered saline solution (sham; $n = 7$) into the medial septum and vertical diagonal band of Broca (ms/vdbb). Results are expressed as mean \pm SEM. (A) Subanesthetic state set by repeated administration of 25 mg/kg propofol. (B) Anesthetic state set by repeated administration of 50 mg/kg propofol. * $P < 0.05$ compared with SHAM-operated animals.

creased, with a more pronounced effect in lesioned animals as compared with the control ones (fig. 4A). At the cumulative dose of 175 mg/kg, we observed a shift from a subanesthetic state to an anesthetic state (*i.e.*, anaesthetic score ≥ 0.6) in SAPO rats but not in SHAM rats. Analysis of variance for repeated measures showed a significant effect of the group factor ($F_{1,17} = 18.20, P < 0.05$), a significant effect of the cumulative dose of propofol ($F_{6,108} = 15.79, P < 0.05$), and a significant interaction between the two factors ($F_{6,108} = 3.6, P < 0.05$).

After the repetitive injection of 50 mg/kg (anesthetic state), the anaesthetic score increased as the cumulative dose of propofol was increased. This effect was more pronounced in lesioned animals as compared with the control ones (fig. 4B). Therefore, the anaesthetic potency

Table 1. Mean Optic Density (Arbitrary Units) of Acetylcholinesterase Reaction Products in Various Brain Structures of Rats that Sustained Injections of 192 Immunoglobulin G–Saporin or Phosphate-buffered Saline Solution

	Hippocampus	Frontal Cortex	Parietal Cortex	Thalamic Reticular Nucleus	Striatum
ICV					
SHAM	0.251 ± 0.016	0.178 ± 0.013	0.144 ± 0.012	0.189 ± 0.013	0.589 ± 0.013
SAPO	0.111 ± 0.016*	0.058 ± 0.007*	0.066 ± 0.007*	0.199 ± 0.016	0.552 ± 0.025
NBM					
SHAM	0.160 ± 0.09	0.110 ± 0.008	0.085 ± 0.005	0.143 ± 0.005	0.374 ± 0.007
SAPO	0.142 ± 0.006	0.062 ± 0.004*	0.042 ± 0.003*	0.124 ± 0.005†	0.359 ± 0.020
MS/vDBB					
SHAM	0.226 ± 0.027	0.123 ± 0.018	0.122 ± 0.013	0.279 ± 0.018	0.567 ± 0.021
SAPO	0.070 ± 0.010*	0.049 ± 0.007*	0.096 ± 0.011	0.242 ± 0.017	0.573 ± 0.014

Data are presented as mean ± SEM in sham-operated and lesioned rats.

* $P < 0.0001$. † $P < 0.05$.

ICV = intracerebroventricular; MS/vDBB = medial septum and vertical diagonal band of Broca; NBM = nucleus basalis magnocellularis; SAPO = injection of 192 immunoglobulin G–saporin; SHAM = injection of phosphate-buffered saline solution.

of propofol was significantly increased in SAPO rats as compared with SHAM rats. Analysis of variance for repeated measures showed a significant effect of the group factor ($F_{1,17} = 18.20$, $P < 0.05$) and of the cumulative dose of propofol ($F_{4,68} = 1.19$, $P < 0.05$).

Histologic Analyses

The multivariate analysis of variance showed a main significant effect of SAPO *versus* SHAM ($F_{1,55} = 14.60$, $P < 10^{-4}$) and a significant interaction between sites of injection (intracerebroventricular, NBM, MS/vDBB), brain areas, and SAPO *versus* SHAM ($F_{8,220} = 3.89$, $P < 10^{-4}$) on the acetylcholinesterase optic densities. When compared with SHAM, intracerebroventricular lesioned rats exhibited significant reductions of the acetylcholinesterase optic densities in parietal (–54% on average) and frontal (–68% on average) cortices and in the hippocampus (–56% on average) (all $P < 0.05$). The acetylcholinesterase optic density was not significantly modified by the 192 IgG-saporin in the striatum and the thalamic reticular nucleus (table 1).

When the 192 IgG-saporin was injected into the NBM, the acetylcholinesterase optic density was significantly decreased in the frontal cortex (–43% on average) and in the parietal cortex (–50% on average) (all $P < 0.05$). The acetylcholinesterase optic density, however, was not significantly modified by 192 IgG-saporin in the hippocampus, the striatum, or the thalamic reticular nucleus.

Finally, when 192 IgG-saporin was injected into the MS/vDBB, there was a significant decrease in the acetylcholinesterase optic density in the hippocampus (–69% on average) and in the frontal cortex (–60% on average) (all $P < 0.05$). In the other anatomical structures assessed, we did not observe a statistically significant modification of the staining density of acetylcholinesterase.

Examples of density and distribution of acetylcholinesterase reaction products are shown in figure 5.

Discussion

Our results show that a general basal forebrain cholinergic depletion induced by intracerebroventricular injection

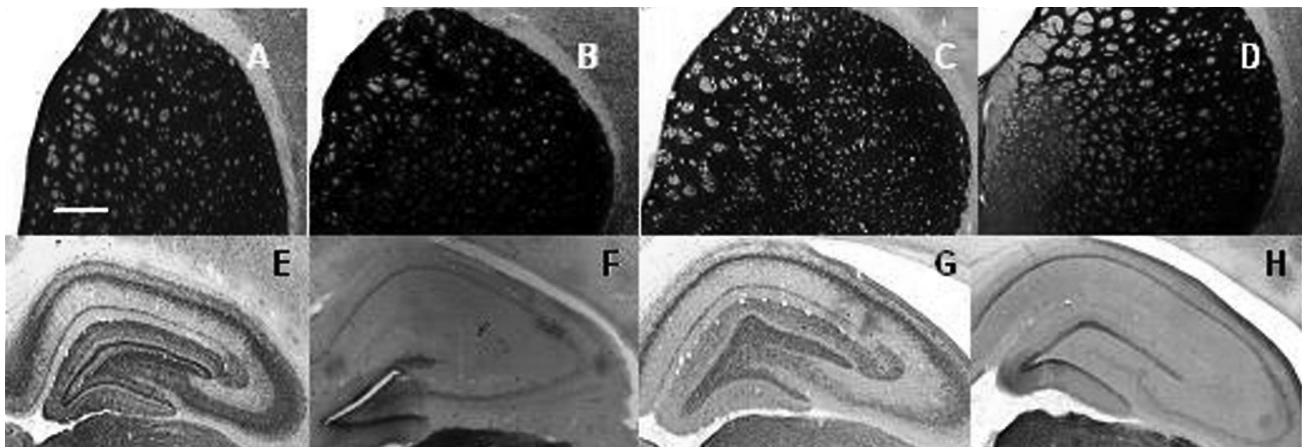


Fig. 5. Typical examples of acetylcholinesterase staining on coronal brain sections through the striatum (A–D) and the hippocampus (E–H) in rats subjected to sham operation (A and E), intracerebroventricular injection of 192 immunoglobulin G–saporin (B and F), or intraparenchymal injection of 192 immunoglobulin G–saporin, either into the nucleus basalis magnocellularis (C and G) or into the medial septum and vertical diagonal band of Broca (D and H). Note the relative preservation of acetylcholinesterase reaction products in the striatum (A–D) and the important depletion in the hippocampus (F and H). Scale bar = 530 μm .

tions of 192 IgG-saporin (experiment 1) modulates the anesthetic effects of propofol in rats. The total cumulative dose of propofol required to shift from a subanesthetic state to an anesthetic one was lower in the lesioned rats than in the control rats. Moreover, the anesthetic potency was significantly increased in the rats that had sustained intracerebroventricular injections of 192 IgG-saporin as compared with the controls. In a previous study based on a comparable basal forebrain extent of cholinergic depletion (intracerebroventricular injection), we observed that the sedative effect of propofol, as assessed by evaluation of the locomotor effects of the drug, was alleviated in rats subjected to basal forebrain cholinergic depletion.²⁰ At first glance, these results seem contradictory with the current ones. However, in the current experiment, we observed that the anesthetic score found after the first injection of 25 mg/kg (a dose close to that used in our previous study, namely 30 mg/kg) was lower in the lesioned animals than in the controls (fig. 2A), even though this difference did not reach statistical significance. The scale used in the current study to determine the anesthetic score is a sensitive one and is well adapted to studying the modulation of anesthetic potency after pharmacologic alteration of a specific neurotransmitter system.³² After combining the current results on anesthetic effects with the previous ones on locomotor impairment,²⁰ it becomes clear that the basal forebrain cholinergic system modulates the effects of propofol. However, the interactions seem to depend on the various effects induced by propofol. A basal forebrain cholinergic depletion (intracerebroventricular injection) alleviates the locomotor impairment induced by low doses of propofol, facilitates the shift from a subanesthetic state to an anesthetic state, and potentiates the anesthetic state induced by propofol.

More and more, the pharmacologic mechanisms sustaining different effects of propofol seem to differ according to the brain area.^{1,8} Recent findings in humans evidenced that the cortex and subcortical structures have different sensitivities to propofol.³³ Our current results combined with our previously reported ones²⁰ are in accord with the concept that, within the brain, the mechanisms of action of an anesthetic might differ from one brain area to another, which could *in fine* have a dose-dependent incidence on the type of behavioral effect elicited by the anesthetic.

Importantly for locomotion and movement, no noticeable cholinergic depletion in the adjacent striatum was observed in any experiment, an observation that is in accord with previous reports.^{20,22} When 192 IgG-saporin is injected into the ventricles, the immunotoxin reaches other target areas than the basal forebrain, in particular the cerebellar Purkinje cells. No disturbance of spontaneous behavior and locomotor activity has been observed in rats after administration of 192 IgG-saporin into the lateral ventricles, the NBM, and the MS/vDBB.^{22,27,29,30} However,

intracerebroventricular injection of 192 IgG-saporin as well as NBM injection may result in a motor coordination impairment.²⁶ One could argue that our behavioral assessment of anesthetic potency is contaminated by such sensorimotor biases. This is not the case. We observed the same potentiation of anesthetic potency of propofol for intracerebroventricular and MS/vDBB injection, whereas the injection of 192 IgG-saporin into the MS/vDBB has no impact on motor coordination impairment.^{29,30}

Cholinergic neurons represent a portion of the cortically projecting and other basal forebrain neurons, which include GABAergic neurons and also various monoaminergic terminals or neurons.^{30,34} By increasing discharges and firing in rhythmic bursts, cholinergic neurons can thus stimulate cortical activity. Colocalized GABAergic basal forebrain neurons oppose these actions. Previous studies have evidenced that the local injection of a GABAergic agonist (muscimol) in different pathways of the basal forebrain increased the potency of both volatile (halothane and isoflurane) and nonvolatile (propofol and pentobarbital) anesthetics.^{1,2} The immunotoxin 192 IgG-saporin is highly selective for cholinergic neurons,²¹⁻³⁰ and cholinergic compensations are extremely weak or inexistent.³⁵ Some limited consequences of the immunotoxin on GABAergic neurons have been described using high to very high doses of 192 IgG-saporin (above 1 μ g). As a compensatory mechanism to the cholinergic lesion in the septum, both monoaminergic and serotonergic sprouting have been observed, but only several months after surgery. At the dose of 192 IgG-saporin used in the current study and at the delay between surgery and behavioral assessment (4 weeks), the lesions performed do not modify noncholinergic markers in various brain areas, including forebrain nuclei or targets.³⁰

The cholinergic activation of the cortex involved in the maintenance of consciousness relies on different pathways, the thalamocortical pathway, namely the cortical projections from the NBM and the hippocampocortical connections.³⁶ The injection of 192 IgG-saporin into the NBM resulted in a cholinergic depletion of the frontal and parietal cortices (approximately 40-50%) and a depletion of the thalamic reticular nucleus (approximately 13%) that was weak but of statistical significance. Such a cholinergic depletion was associated with a slight increase of the anesthetic potency of propofol in rats that underwent the NBM lesion. Indeed, the difference was present from the lowest dose onward and remained constant throughout the cumulative dose (fig. 3B). Moreover, the total cumulative dose of propofol required to shift from a subanesthetic state to an anesthetic one was not modified for these animals (fig. 3A). Based on our results, it is not possible to conclude about the respective involvement of the direct cholinergic cortical or the thalamic projections from the NBM. The

depletion of approximately 13% in the thalamic reticular nucleus seemed low compared with the 40–50% depletion in the frontal and parietal cortices, but it might be sufficient to modify the anesthetic potency of propofol *via* the disruption of the thalamocortical pathways. Our results evidenced some involvement of the cholinergic projections from the NBM in mediating the anesthetic potency of propofol, but such involvement seemed to differ according to the extent of the cholinergic depletion relying on the site of injection of the immunotoxin (intracerebroventricular, NBM, and MS/vDBB).

Interestingly, with regard to the cholinergic local pathways, it must be noted that the cholinergic lesion of the septohippocampal pathway has a more pronounced effect in the modulation of the anesthetic potency than the lesion of the NBM. The anesthetic potency of propofol was significantly increased in the rats that had been subjected to NBM (experiment 2) or MS/vDBB (experiment 3) lesions, but the total cumulative dose of propofol required to shift from a subanesthetic state to an anesthetic one was decreased only after MS/vDBB lesions. The injection of 192 IgG-saporin into the MS/vDBB resulted in a dramatic cholinergic depletion in the hippocampus (approximately 70%), but also in the frontal cortex (approximately 60%), but there was no modification of acetylcholinesterase optic density in the thalamic reticular nucleus (as observed for NBM lesion). Histochemical evaluation of the effects of the injections of 192 IgG-saporin into the MS/vDBB confirmed previous observations that this injection produces a dramatic depletion of acetylcholinesterase reaction products in the hippocampus (ranging from 60% to 90% across data from the literature).^{23,27} Importantly, we observed a similar cholinergic depletion of the hippocampus after either the intracerebroventricular or the MS/vDBB injection, and similar effects on both anesthetic state setting and anesthetic potency of propofol. Intracerebroventricular and intra-MS/vDBB but not intra-NBM injection resulted in cholinergic depletion of hippocampus in the current study. Our results suggest that besides cholinergic cortical projections from the basal forebrain, the hippocampocortical pathway plays a role in mediating the anesthetic effects of propofol.

Recent research on the anatomical sites responsible for general anesthesia pointed out that general anesthetics must modify neuronal activity in more than a single brain area simultaneously, and do so in a coordinated manner to produce loss of consciousness and unresponsiveness. How may the septohippocampal system participate in general anesthesia? First, the current hippocampal literature suggests that activity of the ascending brain stem pathways supplies the hippocampus with sensory information relevant to the initiation of movement. In the framework of the sensorimotor integrative model of the hippocampus,³⁷ the MS functions are the node in the ascending pathways, sending both cholin-

ergic and GABAergic projections to the hippocampus, the generation of hippocampal θ band oscillation, and synchrony being related to sensorimotor processing and integration. Second, connection of the septohippocampal pathway with the mesopontine cholinergic neurons may help to maintain overall vigilance and influence the sleep-wake state.^{38,39} The anesthetic state is associated with a θ field firing decline in the hippocampus.⁴⁰ Through the above pathways, a general anesthetic might suppress awareness and movement control. Complete inactivation of the septohippocampal outputs, either GABAergic ones as shown in the previous study² or cholinergic ones as in the current study, may facilitate loss of consciousness and unresponsiveness and may potentiate general anesthesia.

In summary, the injection of the 192 IgG-saporin into the MS or in the cerebral ventricles produced a lesion that preferentially damaged the cholinergic innervation of the hippocampus and the frontal cortex, as previously described. Such a cholinergic disruption was responsible for an increased potency of propofol anesthesia in rats. Our results suggest that the basal forebrain cholinergic system could be a modulator (direct or indirect) for the setting and potency of an anesthetic state. Besides cortical cholinergic basal forebrain projections (as from NBM), the septohippocampal system seemed to be involved specifically in mediating general anesthesia. However, to which extent cholinergic basal forebrain pathways participate in the effects of other nonvolatile or volatile anesthetics remains to be determined and is currently under examination.

The authors thank Benjamin Eisenmann (Assistant Engineer, master's thesis in Biology, Groupe de Recherche Expérimentale sur les Répercussions Cognitivo-affectives de l'anesthésie, Inserm U666, Strasbourg, France) for his technical support in analyzing histochemistry.

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