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Biochemical and Electrophysiologic Evidence That Propofol Enhances GABAergic Transmission in the Rat Brain

V. A. Peduto, M.D., A. Concas, Ph.D.,† G. Santoro, Ph.D.,† G. Biggio, Ph.D.,† G. L. Gessa, M.D.‡*

The influence of propofol, a new intravenous anesthetic agent, on brain γ-aminobutyric acid (GABA)-ergic transmission has been investigated both *in vitro* and *in vivo*. *In vitro*, propofol, like benzodiazepines, 1) markedly enhanced ^{3}H-GABA binding in cortical membrane preparations; 2) potentiated muscimol-induced stimulation of $^{36}Cl^{-}$ uptake in membrane vesicle preparations (the propofol potentiating effect was antagonized by bicuculline); and 3) inhibited ^{35}S-TBPS binding to unwashed membrane preparations from rat cerebral cortex. Finally, propofol failed to displace ^{3}H-flunitrazepam from its binding site, indicating that its site of action in brain is different from that of benzodiazepines. *In vivo*, the effect of propofol was studied using single-unit recording of the electrical activity of both nondopaminergic neurons in the pars reticulata of the substantia nigra (PR neurons) and of dopaminergic neurons in the pars compacta of the substantia nigra (DA neurons). PR neurons are known to be inhibited by GABA-mimetic drugs and benzodiazepines, whereas DA neurons are tonically inhibited by PR neurons. The intravenous administration of propofol, in a fat emulsion formulation, produced a brief dose-dependent inhibition of the firing rate of PR neurons. The dose producing 50% inhibition of the firing rate was calculated to be 1.2 ± 0.1 mg/kg. The inhibitory effect lasted less than 5 min. Repeated injections of propofol reproduced the same inhibitory response, whereas continuous infusion (0.5 $mg \cdot kg^{-1} \cdot min^{-1}$) produced a persistent inhibition of neuronal firing. The inhibitory effect of propofol on PR neurons was potentiated by diazepam and reversed by picrotoxin and bicuculline but was not influenced by the benzodiazepine antagonist Ro 15-1788. These findings suggest that propofol exerts a GABA-mimetic action on PR neurons by acting on a site distinct from the benzodiazepine recognition site. Unlike benzodiazepines, propofol inhibited the firing rate of DA neurons with a potency proportional to its inhibitory effect on PR neurons. The inhibition of DA neurons was reversed by bicuculline and picrotoxin. The results suggest that propofol enhances the inhibitory control over DA neurons by strionigral GABAergic neurons. (Key words: Anesthetics, intravenous: propofol; diazepam. Receptors: benzodiazepine; GABA. Neurons: dopaminergic; nondopaminergic.)

AFTER ITS INTRODUCTION into clinical practice, about 4 yr ago, propofol (2,6-diisopropylphenol, Diprivan®), a sterically hindered phenol derivative dissolved in an emulsion formulation, has been widely used as an intravenous anesthetic agent because of its short recovery time either after a single bolus injection or after an infusion lasting even several days.[1–7]

The pharmacology of propofol has been broadly reviewed with regard to its pharmacokinetic and pharmacodynamic properties,[8–11] but little is known of its neurochemical mechanisms of action. In preliminary experiments using bovine adrenomedullary chromaffin cells (which have been shown to possess γ-aminobutyric acid A [$GABA_A$] receptors), Hales and Lambert[12] documented that propofol reversibly and in a dose-dependent manner potentiated the amplitude of membrane currents elicited by locally applied GABA. Subsequently, Collins[13] confirmed that propofol enhances GABA-evoked neuronal inhibition in rat olfactory cortex slices. The ability to potentiate the GABA-induced responses both in central neurons and in bovine chromaffin cells is believed to be a general feature of many anesthetic drugs, such as alphaxalone,[14] barbiturates, and benzodiazepines.[15–17] Apparently by acting at different binding sites, these drugs can modulate the activity of $GABA_A$ receptors and, in addition, can directly activate the chloride channel functionally linked to the $GABA_A$ receptor.[18,19] An enhancement of GABA-mediated chloride ion conductance in some neuronal systems may in part be responsible for their sedative, anticonvulsant, hypnotic, and anesthetic effects.[20,21]

The present study was designed to clarify the influence of propofol on GABAergic transmission. For this purpose, both *in vivo* and *in vitro* experimental models were used.

In order to clarify the molecular mechanism involved in the facilitative action of propofol on GABAergic transmission, we examined the effect of this drug on GABA and benzodiazepine recognition sites and directly on chloride channel function by measuring $^{36}Cl^{-}$ uptake and the binding of t-[^{35}S]butylbicyclophosphorothionate (^{35}S-TBPS). This binding constitutes a very sensitive tool for studying the function of the GABA-dependent chloride channel. In fact, the binding of ^{35}S-TBPS to the recognition sites associated to the $GABA_A$ receptor complex is modulated in an opposite manner by different compounds that specifically enhance (benzodiazepines and barbiturates) and inhibit (bicuculline and β carbolines) the function of the GABA-dependent chloride channel.[22–25]

* Institute of Anesthesia and Intensive Care.

† Chair of Pharmacology, Department of Experimental Biology.

‡ Department of Neuroscience Bernard B. Brodie, University of Cagliari, Italy.

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Address reprint requests to Dr. Gessa: Department of Neuroscience Bernard B. Brodie, University of Cagliari, Via Porcell 4, 09124 Cagliari, Italy.

Furthermore, *in vivo* we studied the effect of propofol on single-unit electrical activities of both nondopaminergic neurons in the substantia nigra pars reticulata (PR neurons) and dopaminergic neurons in the pars compacta (DA neurons). PR neurons are extremely sensitive to inhibitory influences of GABA and GABA-mimetic drugs.[26] In turn, DA neurons are tonically inhibited by PR neurons.[27]

Materials and Methods

In Vitro Experiments

Male adult Sprague-Dawley CD® rats (Charles River, Como, Italy) weighing 180–200 g were used. The animals were kept under a 12 h light–dark cycle at 23 ± 2° C with water and standard laboratory food *ad libitum*. The animals were killed by decapitation in the middle of the light phase. The brains were rapidly removed and the cerebral cortex was dissected out and was used for measurement of $^{36}Cl^-$ uptake, ^{35}S-TBPS binding, ^{3}H-GABA binding, and ^{3}H-flunitrazepam binding.

$^{36}Cl^-$ Uptake

Membrane vesicles were prepared from the cerebral cortex as described by Concas *et al.*[28] Approximately 1 g tissue was homogenized in 7 ml buffer (0° C) containing 20 mM Hepes-Tris, 118 mM NaCl, 4.7 mM KCl, 1.18 mM $MgSO_4$, 2.5 mM $CaCl_2$, and 10 mM D-glucose (*p*H 7.4) using a glass–glass homogenizer (12 strokes). The homogenate was brought to 30 ml with ice-cold buffer and centrifuged at 1,000 × g for 15 min. After discarding the supernatant the pellet was washed once more with the same volume of buffer followed by centrifugation at 1,000 × g for 15 min. The pellet was gently resuspended in buffer to a final protein concentration of 16 mg/ml. Aliquots of tissue (100 μl) were preincubated for 10 min at 30° C prior to the simultaneous addition of 100 μl $^{36}Cl^-$ (2.0 μCi/ml) and muscimol (5 μM). Final incubation volume was 500 μl. Drugs were preincubated with tissue at 30° C for 10 min. Uptake of $^{36}Cl^-$ was terminated 5 s later by the addition of 3.5 ml ice-cold buffer, followed by filtration through 2.5 cm Whatman GF/C filters (presoaked with 0.05% polyethyleneimine to reduce nonspecific binding), using a Hoefer manifold (Hoefer Scientific, San Francisco, CA). The filters were washed twice with 3.5 ml cold buffer, and the $^{36}Cl^-$ content of the filters was determined by liquid scintillation spectrometry. The amount of $^{36}Cl^-$ bound to the filters in the absence of membranes was subtracted from all values.

^{35}S-TBPS Binding

According to the method of Concas *et al.*,[28] cerebral cortices were homogenized with a Polytron PT 10 (setting 5, for 20 s) in 50 volumes of ice-cold 50 mM Tris-citrate buffer (*p*H 7.4 at 25° C) containing 100 mM NaCl. The homogenate was centrifuged at 20,000 × g for 20 min and reconstituted in 50 volumes of Tris-citrate buffer for the binding assay.

^{35}S-TBPS binding was determined in a final volume of 500 μl, consisting of 200 μl tissue homogenate (0.2–0.25 mg protein), 50 μl ^{35}S-TBPS, 50 μl 2 M NaCl, 50 μl drugs or solvent, and buffer to volume. Incubations (25° C) were initiated by the addition of tissue homogenate and were terminated 90 min later (steady state[25,26]) by rapid filtration through glass fiber filter strips (Whatman GF/B). The filters were rinsed with two 4-ml portions of ice-cold Tris-citrate buffer with a Cell Harvester filtration manifold (model M-24, Brandel). Radioactivity bound to the filter was quantified by liquid scintillation spectrometry. Nonspecific binding was defined as binding in the presence of 100 μM picrotoxin. Under these conditions, the specific binding was 85–90% of total binding. Saturation analysis was based on eight different concentrations of ligand (2.5–500 nM). The amount of radioligand was kept constant at 2.5 nM and then diluted with unlabeled TBPS. The maximum binding site density (B_{max}, picomoles per milligram of protein) and the dissociation constant (K_d, nanomolar) were determined by Scatchard analysis.

^{3}H-GABA Binding

According to the method of Biggio,[29] cerebral cortices were homogenized with a Polytron PT 10 (setting 5, for 30 s) in 10 volumes of ice-cold water and centrifuged 10 min at 48,000 × g at 0° C. The pellet was washed once by resuspension and recentrifugation in 10 volumes of 20 mM K_2HPO_4/KH_2PO_4 buffer (*p*H 7.4) containing 50 mM KCl. The membranes were stored at −20° C until used 1–15 days later. On the day of the assay, the membranes were thawed and centrifuged. The pellet was washed three additional times by resuspension and recentrifugation in ice-cold buffer. The tissue was resuspended in 50 volumes of the same buffer, and 300 μl membrane suspension (250–300 μg protein) was added to plastic minivials. The drugs were added in 50-μl aliquots. The total incubation volume was 500 μl. The incubation (10 min at 4° C; steady state) was started by the addition of ^{3}H-GABA and was stopped by centrifugation of the incubation mixture at 48,000 × g for 10 min. The supernatant was discarded, and the pellet was gently washed twice with 4 ml of ice-cold distilled water and then dissolved in 3 ml scintillation fluid (Atomlight, New England Nuclear). Nonspecific binding was defined as binding in the presence of 1 mM unlabeled GABA. Under these conditions, the specific binding was 75–80% of total binding.

Saturation analysis of ^{3}H-GABA binding was performed using nine different concentrations (20–1200 nM)

of ligand. The amount of radioligand was kept constant at 20 nM and then diluted with unlabeled GABA. Scatchard analysis of the binding data was performed using an iterative computer program in BASIC for IBM-PC computer (LIGAND). The program accepts as input the binding data (bound and bound/free) and determines whether the results from individual Scatchard plots are best fitted by a one- or a two-site model. This analysis provides estimates for K_d and B_{max} values.

^{3}H-Flunitrazepam Binding

Cerebral cortices were homogenized in 50 volumes of ice-cold 50 mM Tris-citrate buffer (*p*H 7.4) plus 100 mM NaCl and centrifuged at 20,000 × g for 20 min. The pellet was reconstituted in 50 volumes of Tris-citrate buffer without salts and used for the binding assay. Aliquots of 200 μl tissue homogenate (0.2–0.25 mg of protein) were incubated in the presence of 0.5 nM ^{3}H-flunitrazepam (specific activity 80 Ci/mmol, New England Nuclear), in a total incubation volume of 500 μl. The drugs were added in 50-μl aliquots. Nonspecific binding was determined in the presence of 5 μM diazepam. Under these conditions, specific binding was 90–95% of total binding.

After 60 min incubation at 0–4°C (steady state), the assay was terminated by rapid filtration through glass-fiber filter strips (Whatman GF/B) that were then rinsed with two 4-ml portions of ice-cold Tris-citrate buffer as previously described. Radioactivity present on the filters was determined by liquid scintillation spectrometry.

Proteins were assayed by the method of Lowry *et al.*[30] using bovine serum albumin as standards.

In Vivo Experiments

These experiments were conducted according to the following protocol, which was reviewed and approved by our University Board.

Male adult Sprague-Dawley CD^R rats (250–300 g, Charles River) were anesthetized with halothane and tracheotomized and intubated. A femoral venous catheter was inserted for intravenous drug administrations. The head of each animal was placed in a David Kopf stereotaxic apparatus. Once surgical procedures were accomplished, the incised tissues and those compressed by the stereotaxic device were infiltrated with mepivacaine 3%. Halothane anesthesia was then discontinued and *d*-tubocurarine (10 mg/kg) was injected intraperitoneally. The lungs were mechanically ventilated (Aika® R60). A heating lamp and circulating water blanket were used to maintain body temperature between 37 and 38°C. During the trial, heart rate and frontal cortex electroencephalogram were also monitored.

Glass "Ω dot" recording microelectrodes, filled with a 2% Pontamine Sky Blue solution in CH_3-COONa 0.5 M, were placed in the substantia nigra pars reticulata, according to Paxinos and Watson atlas[31] coordinates adjusted to the rats used. The electrode tip was 0.5–1.0 μm (outer diameter), and the *in vitro* impedance (measured with direct current in 0.9% NaCl) was 4–6 MΩ.[32] PR neurons were identified according to their electrophysiologic patterns. Identification was then confirmed by histologic examination. Briefly, PR neurons, localized 50–200 μm ventrally to dopaminergic neuronal layer in substantia nigra pars compacta, exhibit a biphasic, spontaneous, brief (1.0–1.5 ms) spike repeated at a nearly regular rate of 15–50 per second. The spikes were integrated every 10 s and recorded for 10 min before drug administrations. Steady-state baseline was considered as 100% activity value. Percentage changes in the firing rate were calculated at the times indicated in the Results section, below.

Glass microelectrodes with the same features were placed in the substantia nigra pars compacta. DA neurons were identified according to the method described by Mereu *et al.*[33] These neurons show a regular firing rate or brief bursting activity (2–5 spikes or bursts per second).

Chemicals

Propofol was kindly provided by ICI-Pharma (Milan, Italy). Diazepam and quazepam were a generous gift from Hoffmann-La Roche (Basel, Switzerland) and from Essex (Milan, Italy), respectively.

For *in vitro* experiments ($^{36}Cl^-$ uptake, ^{35}S-TBPS, and ^{3}H-flunitrazepam binding), stock solutions of propofol (56 mM) and benzodiazepines (10 mM) were dissolved in dimethyl sulfoxide, and serial dilutions were made up in the respective incubation buffer. For ^{3}H-GABA binding, the same concentrations of propofol were prepared using ethanol instead of dimethyl sulfoxide. Control groups received the same amount of solvent. Bicuculline methiodide (Pierce Chemical Co.) was dissolved in buffer.

For *in vivo* experiments propofol was administered in a fat emulsion formulation consisting of soybean oil 100 mg, purified phosphatide egg 12 mg, glycerol 22.5 mg, sodium hydroxide 0.07 mg, and water to 1 ml. The emulsion, if necessary, was diluted with water. Muscimol, picrotoxin HCl (Sigma), and bicuculline (Pierce) were dissolved in saline.

Ro 15-1788 (Hoffman-La Roche) was suspended in a 4% aqueous solution of Tween 80. Diazepam (Hoffman-La Roche) was used in the commercially available solution and diluted in water. All drugs were injected intravenously in a volume of 1 ml/kg, when infused. Propofol was administered in a volume of 1 $ml \cdot kg^{-1} \cdot min^{-1}$.

No effect was observed after administration of the solvents alone.

TABLE 1. Bicuculline Suppresses Propofol-induced Potentiation of Muscimol Effect on $^{36}Cl^-$ Uptake

	$^{36}Cl^-$ Uptake (nmol per mg protein per 5 s)	
	Basal	+ Muscimol (5 μM)
Solvent	11.7 ± 0.9	22.8 ± 1.8*
Propofol 30 μM	10.5 ± 1.2	32.9 ± 2.2†
Bicuculline 50 μM	9.4 ± 1.4	10.6 ± 1.6†
Bicuculline 50 μM + propofol 30 μM	10.0 ± 1.8	12.3 ± 1.5†

Membrane vesicles were preincubated for 10 min at 30° C in the presence of propofol, bicuculline, or solvent before the addition of $^{36}Cl^-$ and muscimol. Data are the mean ± SEM of four experiments performed in quadruplicate.

* $P < 0.01$ *versus* basal.

† $P < 0.05$ *versus* muscimol.

Results

In Vitro Experiments

As shown in table 1, propofol *per se* failed to affect unstimulated $^{36}Cl^-$ uptake in membrane vesicles preparations from rat cerebral cortex, but enhanced in a dose-dependent manner, like diazepam and quazepam, the stimulation of chloride channel conductance induced by muscimol, a GABA-mimetic drug (fig. 1). However, while the efficacy of propofol was similar to that of quazepam, the former showed a lower potency with respect to diazepam and quazepam. Moreover, propofol (10 μM) shifted the concentration–response curve for muscimol (1–100 μM) on $^{36}Cl^-$ uptake to the left without a change in its maximum (fig. 2). Propofol-induced increase of muscimol-activated $^{36}Cl^-$ uptake was antagonized by bicuculline, a selective GABA-receptor blocking drug[34] (table 1).

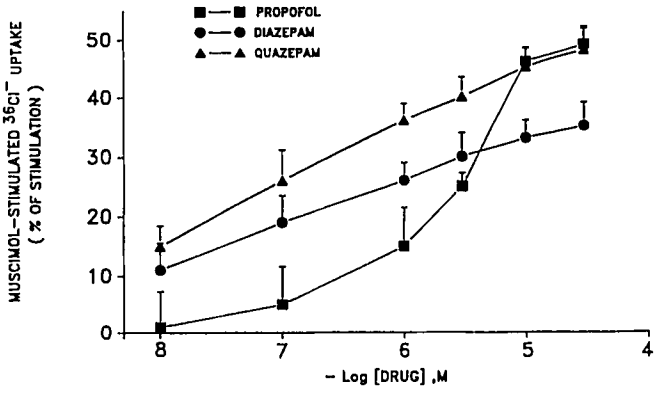

FIG. 1. Propofol, like diazepam and quazepam, potentiates muscimol-induced stimulation of $^{36}Cl^-$ uptake in the rat cerebral cortex. Membrane vesicles were preincubated for 10 min in the presence of drugs before the addition of $^{36}Cl^-$ + muscimol (5 μM). Data are the mean ± SE of four different experiments, each run in quadruplicate.

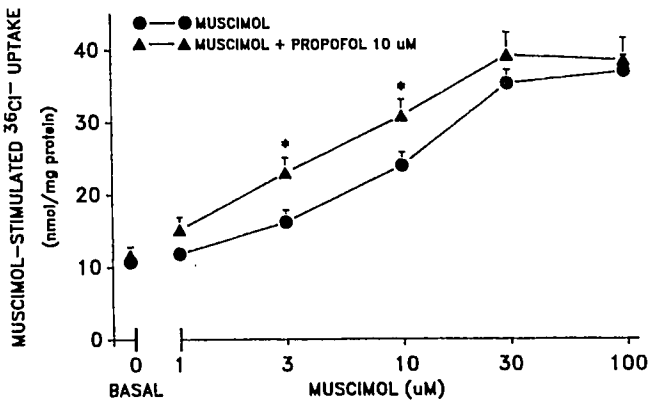

FIG. 2. Effect of propofol on the concentration–response curve for muscimol of $^{36}Cl^-$ uptake by membrane vesicles from rat cerebral cortex. Membrane vesicles were preincubated for 10 min in the presence of propofol or solvent before the addition of $^{36}Cl^-$ + muscimol. Data are the mean ± SE of four different experiments each run in quadruplicate. *$P < 0.05$ *versus* muscimol.

To investigate whether the effect of propofol on the chloride channel was mediated by a direct interaction of propofol with the GABA or benzodiazepine recognition sites, we studied the effect of this compound on ^{3}H-GABA and ^{3}H-flunitrazepam binding in the rat cerebral cortex.

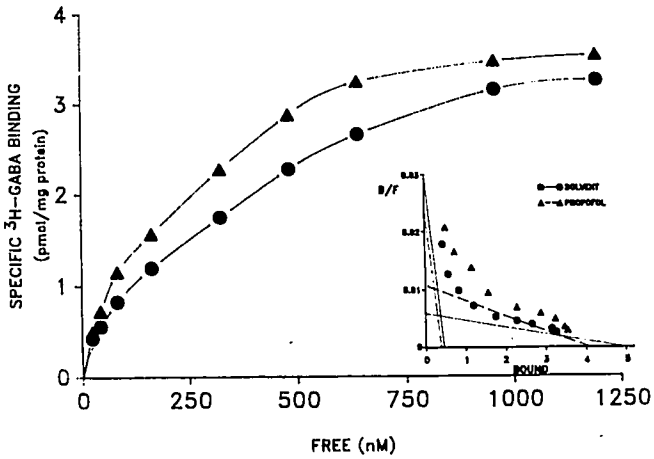

FIG. 3. Saturation isotherm of ^{3}H-GABA binding in the absence and in the presence of propofol (10 μM). *Inset:* Scatchard plots are the transformation of saturation experiments using nine concentrations of ^{3}H-GABA (20–1200 nM). The figure shows a typical experiment that was replicated five more times with very similar results. The B_{max} (picomoles per milligram protein) and K_d (nanomolar) values for each experimental group were as follows (mean ± SEM of five independent experiments): solvent: $B_{max_1} = 0.41 \pm 0.05$, $B_{max_2} = 5.06 \pm 0.7$; $K_{d1} = 14 \pm 2$, $K_{d2} = 818 \pm 51$; propofol: $B_{max_1} = 0.38 \pm 0.06$; $B_{max_2} = 4.33 \pm 0.5$; $K_{d1} = 16 \pm 4$, $K_{d2} = 401 \pm 26$*. *$P < 0.001$ *versus* solvent.

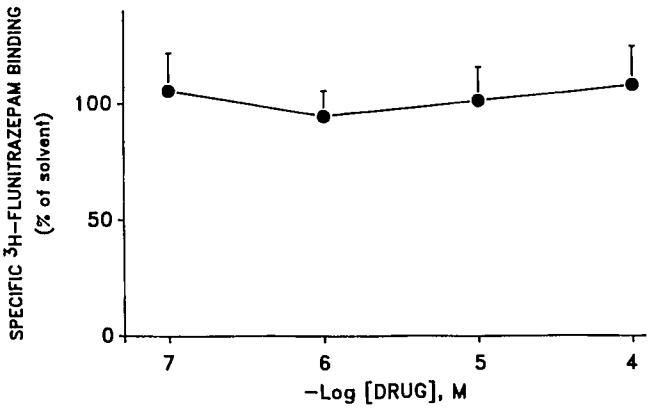

FIG. 4. Failure of propofol to modify specific ^{3}H-flunitrazepam binding in the rat cerebral cortex. Membranes were incubated for 60 min at 0° C in the presence of 0.5 nM ^{3}H-flunitrazepam. Data are the mean ± SE of three experiments. Specific binding of ^{3}H-flunitrazepam in the control group was 205 ± 2 fmol/mg protein (n = 3) and was 90–95% of total binding.

As shown in figure 3, nonlinear regression analysis of binding data from saturation analysis of ^{3}H-GABA binding yielded curvilinear Scatchard plots that were best fitted by a two-site model—a model with both high- and low-affinity components. Propofol produced a marked increase in the affinity of the low-affinity ^{3}H-GABA binding sites (as indicated by the lower dissociation constant K_{d2}) without a significant change in the maximum number of binding sites (B_{max}^2) or in the high-affinity parameters (K_{d1} and B_{max}^1). In contrast, propofol failed to displace ^{3}H-flunitrazepam from its binding sites, even in high concentrations (fig. 4).

Taken together, these results exclude a competitive interaction of propofol with GABA or benzodiazepine recognition sites.

Finally, to further clarify the mechanism of propofol-induced potentiation of GABA response, we evaluated its effect on ^{35}S-TBPS binding to chloride-channel protein coupled to $GABA_A$ receptor. On this subject we studied the action of this drug in unwashed membrane preparations from rat cerebral cortex, which are believed to be rich in endogenous GABA. In a preliminary report[35] we showed that propofol induced a concentration-dependent decrease of ^{35}S-TBPSbinding. The effect is similar to that induced by other general anesthetics (steroids and barbiturates). Saturation analysis of ^{35}S-TBPS binding indicated that propofol inhibits ^{35}S-TBPS binding in an allosteric rather than in a competitive manner. In fact, both

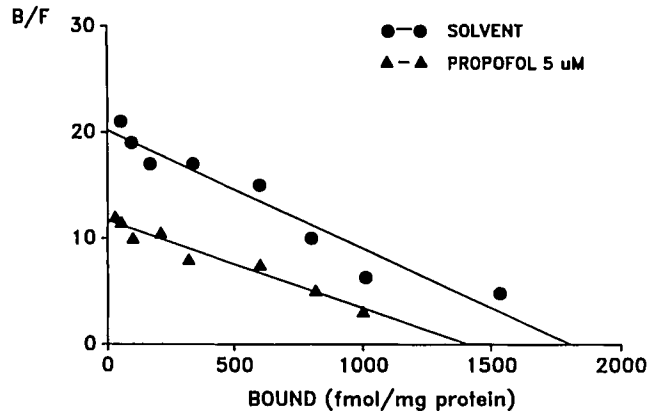

FIG. 5. Effect of propofol on ^{35}S-TBPS binding to cortical membranes from rat cerebral cortex. Scatchard plots are the transformation of saturation experiments using eight concentrations of ^{35}S-TBPS (2.5–500 nM). The figure shows a typical experiment that was replicated four more times with very similar results. The B_{max} (picomoles per milligram protein) and K_D (nanomolar) values for each experimental group were as follows (mean ± SEM of five independent experiments): solvent B_{max} = 1.76 ± 0.11, K_d = 93 ± 6; propofol B_{max} = 1.36 ± 0.09*; K_d = 121 ± 8.* *$P < 0.05$ *versus* control.

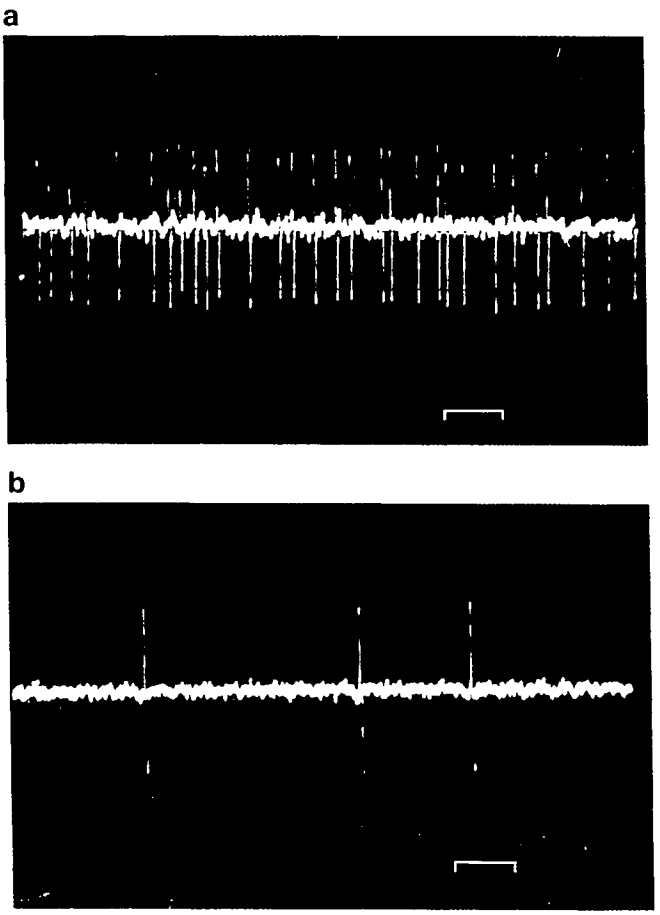

FIG. 6. Scope traces depicting a pars reticulata neuron firing spontaneously at a frequency of 60 Hz. *A*: Before the intravenous administration of propofol (5 mg/kg), *B*: Thirty seconds after propofol, spontaneous activity is drastically inhibited to 6 Hz. Space bar = 50 ms.

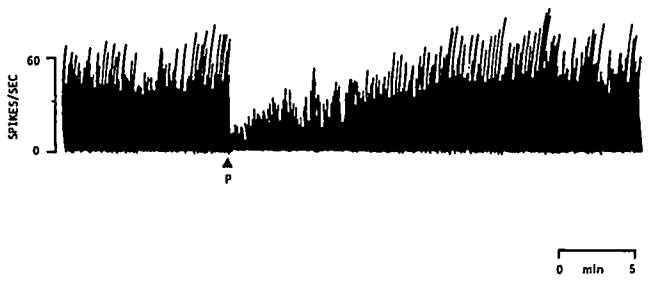

FIG. 7. Typical example of the inhibitory effect of propofol (P) (5 mg/kg) on the electrical activity of a PR-neuron.

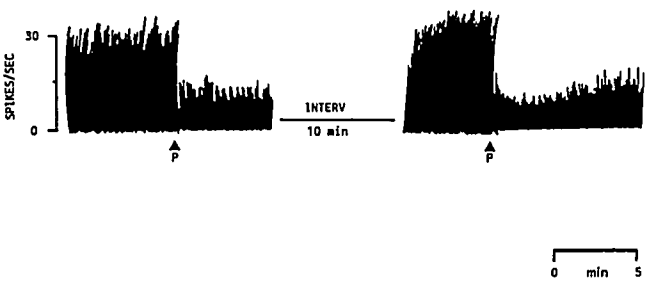

FIG. 9. Typical example of the inhibitory response to a second dose of propofol (P) of a PR neuron. Results from four experiments showed that the mean (± SEM) percent inhibition produced by a first and a second (about 10 min apart) injection of propofol at the dose of 5 mg/kg was 78.3 ± 23.1 and 83.6 ± 19.3, respectively.

the apparent K_d and B_{max} of ^{35}S-TBPS binding were altered by propofol (fig. 5).

In Vivo Experiments

The intravenous administration of propofol inhibited the firing rate of PR neurons (fig. 6). Of the 27 neurons tested, only 4 were found to be insensitive to the inhibitory effect of propofol. As shown in figure 7, propofol-induced inhibition of firing had a rapid onset and a short duration.

The cumulative dose–response curves for the inhibition of PR neurons induced by propofol, diazepam, and muscimol were compared. As shown in figure 8, propofol-induced maximal inhibition of firing was greater than that produced by diazepam and comparable to that of muscimol.

The ED_{50} of propofol was calculated to be 1.2 ± 0.1 mg/kg.

As shown in figure 9, the administration of a second dose of propofol, when the effect of previous one had just lapsed, produced similar inhibitory response.

As shown in figure 10, a continuous propofol infusion (0.5 $mg \cdot kg^{-1} \cdot min^{-1}$) induced a stable and nearly complete firing inhibition, lasting throughout the infusion period and disappearing within a few minutes after propofol discontinuation.

The inhibitory effect of propofol was readily reversed by picrotoxin (fig. 11 and table 2). Bicuculline was found to reverse propofol-induced inhibition so slowly that it was not easy to distinguish such a reversal from a spontaneous recovery of firing. Therefore, a true antagonistic effect by bicuculline could be demonstrated by testing propofol in the same animal before and after bicuculline administration. As shown in table 3, bicuculline at the

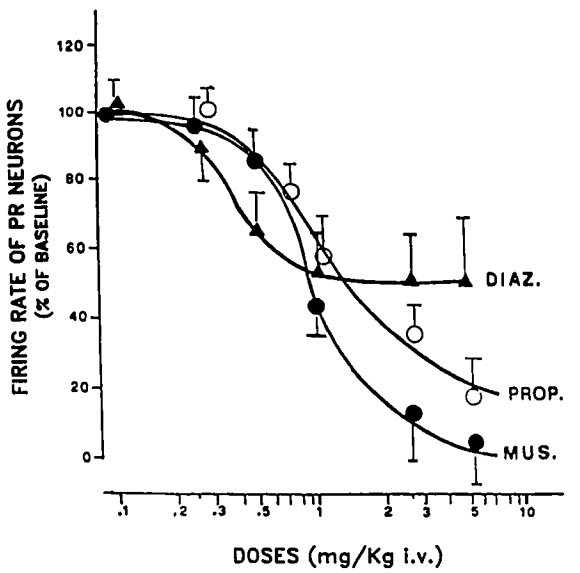

FIG. 8. Dose–response curves of the inhibitory effect elicited by propofol, diazepam and muscimol on the firing rate of PR-neurons. The percent changes in the firing rate were calculated from the mean number of spikes per second counted during the 5 min preceeding the drug injection and the number counted during 1 min at peak drug effect. Each point is the mean (± SEM) obtained from the responses of 6–12 neurons.

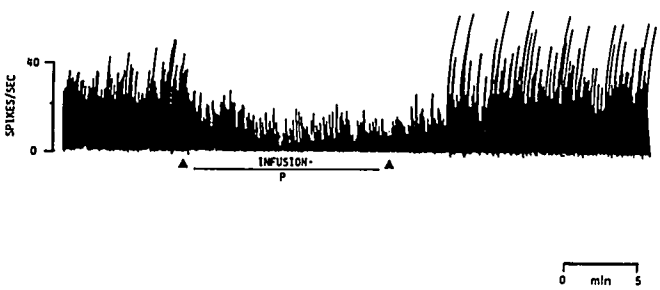

FIG. 10. Typical example of the inhibition produced by a continuous intravenous infusion (0.5 $mg \cdot kg^{-1} \cdot min^{-1}$) of propofol (P) on the firing rate of a PR neuron. When propofol was reinfused to the same animal for 30 min, a similar degree of inhibition was produced and persisted throughout the infusion period. Similar results were obtained in three separate experiments. After interruption of the perfusion the firing rate rapidly returned to the baseline.

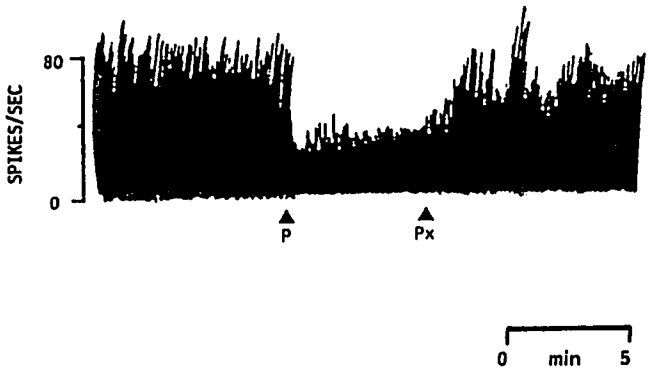

FIG. 11. Typical example of the picrotoxin-induced reversal of the inhibitory effect of propofol on the firing rate of a PR neuron. Propofol (P): 5 mg/kg; pricrotoxin (Px): 4 mg/kg.

dose of 5 mg/kg completely prevented propofol-induced inhibition of firing rate in PR neurons. The effect of propofol was not antagonized by the classic benzodiazepine antagonist Ro 15-1788[36] (results not shown). However, when propofol was administered following a supramaximally active dose of diazepam, it produced a further inhibition of firing rate in PR neurons (fig. 12).

As shown in figure 13, propofol was found to inhibit the firing rate of DA neurons. The effect was dose-related; the ED_{50} was calculated to be 6.8 ± 1.3 mg/Kg; and the inhibitory effect was brief (less than 5 min). As on the PR neurons, the inhibitory effect of propofol on DA neurons was antagonized by picrotoxin and bicuculline (results not shown).

Discussion

The data reported support the view that propofol facilitates GABA-mediated neuronal inhibition.

In particular, the results of *in vitro* experiments indicate that, similarly to benzodiazepines,[29,37-39] propofol increased ^{3}H-GABA binding to cortical membrane preparations and potentiated GABA-mediated opening of the chloride channel. Thus, whereas propofol *per se* failed to modify $^{36}Cl^-$ uptake by membrane vesicles, it enhanced the effect of muscimol, a potent $GABA_A$ receptor agonist. Conversely, bicuculline, a selective $GABA_A$ receptor antagonist,[34] antagonized not only the positive effect of muscimol on $^{36}Cl^-$ uptake but that of propofol as well. The contention that propofol facilitates GABA-mediated opening of the chloride channel is supported by the results on ^{35}S-TBPS binding[35] (fig. 5).

As mentioned above, ^{35}S-TBPS binds to the chloride channel protein, from which it may be displaced by picrotoxin and other convulsant drugs: these drugs, in turn, bind the same chloride channel protein and decrease the frequency and/or duration of the channel opening.[22,23] Paradoxically, also, drugs that increase the frequency and/or the duration of the chloride channel opening, such as benzodiazepines and barbiturates, have been shown to inhibit ^{35}S-TBPS binding.[22-25] However, such inhibition, unlike that of the convulsants, is noncompetitive, being mediated by an allosteric alteration of the ^{35}S-TBPS recognition site. Like benzodiazepines, barbiturates, and anesthetic steroids,[22-25,40,41] propofol was found to inhibit ^{35}S-TBPS binding in a noncompetitive manner. As for the $^{36}Cl^-$ uptake effect, the effect of propofol on ^{35}S-TBPS

TABLE 2. Reversal of Propofol-induced Inhibition of Firing Rate of PR Neurons by Picrotoxin

Treatment	Number of Cells	Firing Rate (% of baseline, mean ± SEM)
Propofol + saline	24	18.6 ± 8.8
Propofol + picrotoxin	6	117.4 ± 21.6*

Picrotoxin 4 mg/kg or saline was given 2 min after propofol 10 mg/kg. Percent changes were calculated from the mean firing rate recorded during the interval between the 2nd and 4th min following propofol injection at peak effect.
* $P < 0.001$, Student's t test.

TABLE 3. Prevention of Propofol-induced Inhibition of Firing Rate of PR Neurons by Bicuculline

Treatment	Firing Rate (% of baseline)	
	First Propofol Injection	Second Propofol Injection
Propofol + saline + propofol	61.3 ± 23.1	54.3 ± 12.6
Propofol + bicuculline + propofol	48.6 ± 27.4	103.1 ± 4.3*

Propofol (5 mg/kg) was injected twice 10 min apart. Two minutes before the second propofol injection, when the firing rate had returned to the normal, bicuculline (5 mg/kg) or saline was injected. Mean firing rate was calculated at 2 min following propofol injection.
* $P < 0.001$, Student's t test.

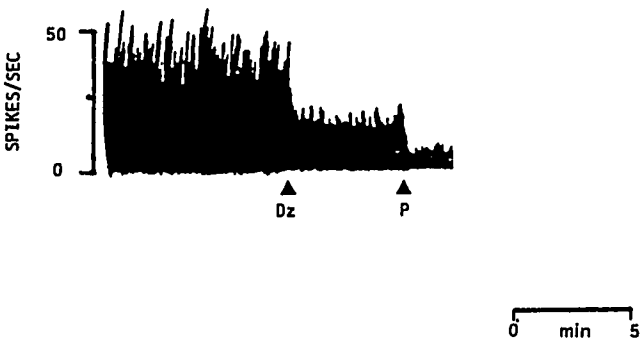

FIG. 12. Propofol (P) (5 mg/kg) further inhibits firing rate of a PR neuron following a supramaximally active dose of diazepam (Dzx) (1 mg/kg). The histogram is a typical example of three experiments.

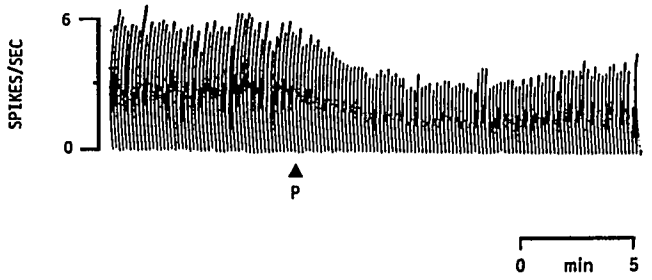

FIG. 13. Inhibition by propofol (P), 5 mg/kg of the firing rate of a DA neuron. The histogram is a typical example of six experiments. The administration of propofol at cumulative doses of 1–10 mg/kg inhibited neuronal firing by 10–90% in a dose-related manner (n = 6).

binding seems to be mediated by a facilitation of the GABA action. In fact, in the washed membrane preparations, from which endogenous GABA was removed, propofol effect on ^{35}S-TBPS binding was lost (results not shown).

However, the site of action of propofol in facilitating a GABA-mediated effect is different from that of benzodiazepines. In fact, *in vitro* propofol failed to displace ^{3}H-flunitrazepam from the binding site, and in *in vivo* experiments the inhibitory effect of propofol on neuronal activity (see below) was not antagonized by flumazenil, the selective benzodiazepine antagonist.[36] Moreover, the binding site of propofol seems to be different from that of GABA, since propofol failed to displace ^{3}H-GABA from its binding site.

Propofol concentrations effective *in vitro* in facilitating GABA-mediated responses are within the concentrations that can be reached after the doses of the drug used in the *in vivo* experiments.[3,42] Therefore, the data from the *in vitro* experiments are relevant in explaining results obtained *in vivo*.

Intravenously administered propofol inhibited the spontaneous firing activity of PR neurons. The inhibitory efficacy of propofol was greater than that of diazepam and comparable to that of muscimol. As expected from the results *in vitro*, propofol-induced inhibition of firing of PR neurons was antagonized by bicuculline, a specific $GABA_A$ antagonist, and by picrotoxin, a drug that binds to a protein subunit of the $GABA_A$ receptor complex and decreases the duration of the channel opening.[43]

Moreover, consistent with the *in vitro* results, we found that the inhibitory response to propofol was not antagonized by flumazenil, which instead, as expected, completely antagonized the effect of diazepam. These data suggest that propofol facilitates GABAergic transmission by interacting with a site distinct from the benzodiazepine recognition site. Such a contention is further supported by the finding that, whereas the diazepam inhibitory effect is self-limiting, propofol is able to suppress completely the electrical activity of PR neurons. Moreover, the inhibitory effect of propofol may add to the partial inhibition obtained with a supramaximal dose of diazepam.

Such a synergism should be taken into account in clinical practice. Indeed, the combination of propofol and benzodiazepines has been shown to cause a prolongation of anesthesia.[44]

The effect of propofol on DA neurons in the substantia nigra differs from that of benzodiazepines, which fail to modify the firing rate of DA neurons in this area and even stimulate that of DA neurons in the ventrotegmental area.[27]

As previously mentioned, DA neurons are tonically inhibited by PR neurons but they also underlie inhibitory influences by striatonigral GABAergic neurons.[26,27,45] It is possible that propofol might preferentially facilitate GABAergic inhibition directly on DA neurons, whereas benzodiazepine might preferentially facilitate the GABAergic inhibitory control over PR neurons so that their tonic inhibitory control over DA neurons is removed.

The effects of propofol on DA and PR neuronal activity are consistent with the idea that propofol has a GABA-mimetic action; however, we cannot rule out the possibility that the observed effects of propofol are a consequence of a global decrease in neuronal excitability.

The data reported may have clinical relevance. Indeed, the calculated ED_{50} of propofol for inhibiting the firing rate of PR neurons is not higher than the range of doses used in clinical practice to obtain anesthesia.[3,41] This is even more relevant considering metabolic differences between the human and the rat.

The inhibitory effect of propofol on neuronal activity has features similar to those of the clinical effect of the drug. Like the anesthetic response, the inhibitory effect on firing has a rapid onset and a short duration. Rapid reversal of neuronal inhibition occurs after discontinuation of propofol infusion; in the same way, in clinical practice, recovery from anesthesia readily occurs faster after propofol than after other intravenous anesthetic agents.[8]

Finally, both in clinical practice and in our experimental conditions, repeated bolus injections fail to produce tachyphylaxis. The finding that propofol inhibits the firing rate of DA neurons may be of theoretical as well as practical importance, in raising the possibility that propofol may be effective in clinical situations in which DA neurons seem to be hyperactive (that is, acute alcohol intoxication, psychostimulant drug overdose, or psychotic agitation).

In conclusion, the present findings suggest GABA-potentiating properties of propofol. Like benzodiazepines and steroid anesthetic agents, propofol facilitates both GABA-receptor interaction and GABA-stimulated chloride channel conductance. However, the site of propofol

action seems to be quite different from benzodiazepine and $GABA_A$ recognition sites. Although the above experimental data do not allow the exact identification of neuronal populations specifically responsible for the sedative, hypnotic, and anesthetic effects of propofol, nevertheless they might clarify the molecular mechanism by which the neuronal inhibition is operated. Although the anesthetic action of propofol appears to be closely correlated to its inhibitory influences on PR neurons, further work is needed to exactly identify the propofol site of action.

Propofol may be a new means for interfering with GABAergic transmission and may be a tool in clarifying the GABA role in sleep–awake mechanisms.

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References

1. Doze VA, Shafer A, White PF: Propofol–nitrous oxide *versus* thiopental–isoflurane–nitrous oxide for general anaesthesia. ANESTHESIOLOGY 69:63–71, 1988
2. Heath PJ, Kennedy DJ, Ogg TW, Dunling C, Gilks WR: Which intravenous induction agent for day surgery? A comparison of propofol, thiopentone, methohexitone and etomidate. Anaesthesia 43:365–368, 1988
3. Galletly DC, Short TG: Total intravenous anaesthesia using propofol infusion: 50 consecutive cases. Anaesth Intensive Care 16: 150–157, 1988
4. Schuttler J, Kloos S, Schwilden H, Stoeckel H: Total intravenous anaesthesia with propofol and alfentanil by computer assisted infusion (abstract). Anaesthesia 43:2S–7S, 1988
5. Shafer A, Doze VA, Shafer SL, White PF: Pharmacokinetics and pharmacodynamics of propofol infusions during general anesthesia. ANESTHESIOLOGY 69:348–356, 1988
6. Mackenzie N, Grant IS: Propofol for intravenous sedation. Anaesthesia 42:3–6, 1987
7. Grounds RM, Lalor JM, Lumley J, Royston D, Morgan M: Propofol infusion for sedation in the intensive care unit: Preliminary report. Br Med J 294:397–400, 1987
8. Langley MS, Heel RC: Propofol: A review of its pharmacodynamic and pharmacokinetic properties and use as an intravenous anaesthetic. Drugs 35:334–372, 1988
9. Sebel PS, Lowdon JD: Propofol: A new intravenous anesthetic. ANESTHESIOLOGY 71:260–277, 1989
10. Skues MA, Prys-Roberts C: The pharmacology of propofol. J Clin Anesth 1:387–400, 1989
11. Dundee JW, Clarke RSJ: Propofol. Eur J Anaesth 6:5–22, 1989
12. Hales TG, Lambert JJ: Modulation of the $GABA_A$ receptor by propofol. Br J Pharmacol 93:84P, 1988
13. Collins GGS: Effects of the anaesthetic 2,6-diisopropyl-phenol on synaptic transmission in the rat olfactory cortex slice. Br J Pharmacol 95:939–949, 1988
14. Cottrell GA, Lambert JJ, Peters JA: Modulation of $GABA_A$ receptor activity by alphaxalone. Br J Pharmacol 90:491–500, 1987
15. Study RE, Barker JL: Diazepam and (−) pentobarbital: Fluctuation analysis reveals different mechanisms for potentiation of γ-aminobutyric acid responses in cultured central neurons. Proc Natl Acad Sci USA 78:7180, 1981
16. Bormann J, Clapham DE: Gamma-amino-butyric acid receptor channels in adrenal chromaffin cells: a patch-clamp study. Proc Natl Acad Sci USA 82:2168–2172, 1985
17. Cottrell GA, Lambert JJ, Peters JA: Chloride currents activated by GABA in cultured bovine chromaffin cells. J Physiol (Lond) 365:90P, 1985
18. Olsen RW, Venter JC: Benzodiazepine/GABA Receptors and Chloride Channels Structural and Functional Properties. New York, Alan R. Liss Inc., 1986
19. Biggio G, Costa E: Chloride Channels and their Modulation by Neurotransmitters and Drugs, Biochemical Psychopharmacology, Volume 45, New York, Raven Press, 1988
20. Scholfield CN: Potentiation of inhibition by general anaesthetics in neurones of the olfactory cortex in vitro. Pflugers Arch 383: 249–255, 1980
21. Harrison NL, Simmonds MA: Modulation of the GABA receptor complex by a steroid anaesthetic. Brain Res 323:287–292, 1984
22. Squires RF, Casida JE, Richardson M, Saederup E: ^{35}S-t-butylbicyclophosphorothionate binds with high affinity to brain-specific sites coupled to γ-aminobutyric acid-A and ion recognition sites. Mol Pharmacol 23:326, 1983
23. Ramanjaneyulu R, Ticku MK: Binding characteristics and interactions of depressant drugs with [^{35}S]t-butylbicyclophosphorothionate, a ligand that binds to the picrotoxinin site. J Neurochem 42:221, 1984
24. Gee KW, Lawrence LJ, Yamamura HI: Modulation of the chloride ionophore by benzodiazepine receptor ligands: influence of γ-aminobutyric acid and ligand efficacy. Mol Pharmacol 30:218, 1986
25. Concas A, Serra M, Atsoggiu T, Biggio G: Foot shock stress and anxiogenic β-carbolines increase ^{35}S-TBPS binding in the rat cerebral cortex, an effect opposite to anxiolytics and γ-aminobutyric acid mimetics. J Neurochem 51:1868, 1988
26. Grace AA, Bunney BS: Opposing effects of striatonigral feedback pathways on midbrain dopamine cell activity. Brain Res 333: 271, 1985
27. O'Brien DP, White FJ: Inhibition of non-dopamine cells in the ventral tegmental area by benzodiazepines: relationship to A10 dopamine cell activity. Eur J Pharmacol 142:343, 1987
28. Concas A, Pepitoni S, Atsoggiu T, Toffano G, Biggio G: Aging reduces the GABA-dependent $^{36}Cl^-$ flux in rat brain membrane vesicles. Life Sci 43:1761, 1988
29. Biggio G: The action of stress, β-carbolines, diazepam and Ro 15-1788 on GABA receptors in the rat brain, Benzodiazepine Recognition Site Ligands: Biochemistry and Pharmacology, Edited by Biggio G, Costa E. New York, Raven Press, 1983, pp 105–117
30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951
31. Paxinos G, Watson C: The rat brain in stereotaxic coordinates. New York, Academic Press, 1982
32. Mereu G, Corda MG, Carcangiu P, Giorgi O, Biggio G: The β-carboline ZK 93423 inhibits reticulata neurons: an effect reversed by benzodiazepine antagonists. Life Sci 40:1423, 1987
33. Mereu G, Fadda F, Gessa GL: Ethanol stimulates the firing rate of nigral dopaminergic neurons in unanesthetized rats. Brain Res 292:63, 1984
34. Curtis DR, Dugan AW, Felix D, Johnston GAR: Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord of the cat. Brain Res 32:69–96, 1971

35. Concas A, Santoro G, Mascia MP, Serra M, Sanna E, Biggio G: The general anesthetic propofol enhances the function of γ-aminobutyric acid-coupled chloride channel in the rat cerebral cortex. J Neurochem 55(6)2135–2138, 1990
36. Hunkeler W, Mohler H, Pieri L, Polc P, Bonetti EP, Cumin R, Schaffner R, Haefely W: Selective antagonists of benzodiazepines. Nature 290:514, 1981
37. Skerritt JH, Johnston GAR: Enhancement of GABA binding by benzodiazepines and related anxiolytics. Eur J Pharmacol 89: 193–198, 1983
38. Obata T, Yamamura HI: The effect of benzodiazepines and β-carbolines on GABA-stimulated chloride influx by membrane vesicles from the rat cerebral cortex. Biochem Biophys Res Commun 141:1, 1986
39. Morrow AL, Suzdak PD, Paul SM: Benzodiazepine, barbiturate, ethanol and hypnotic steroid hormone modulation of GABA-mediated chloride ion transport in rat brain synaptoneurosomes, Chloride Channels and their Modulation by Neurotransmitters and Drugs. Edited by Biggio G, Costa E. New York, Raven Press, 1988, p 247
40. Majewska MD, Harrison NI, Schwartz RD, Barker JL, Paul SM: Steroid hormone metabolites are bariturate-like modulators of the GABA receptor. Science 232:1004, 1986
41. Turner DM, Ransom RW, Yang JS-J, Olsen RW: Steroid anaesthetics and naturally occurring analogs modulate the γ-aminobutyric acid receptor complex at a site distict from barbiturates. J Pharmacol Exp Ther 248:960–966, 1989
42. Cummings GC, Dixon J, Kay NH, Windsor JPW, Major E, Morgan M, Sear JW, Spence AA, Stephenson DK: Dose requirements of ICI 35,868 (propofol, 'Diprivan') in a new formulation for induction of anaesthesia. Anaesthesia 39:1168–1171, 1984
43. Ticku MK, Ban M, Olsen RW: Binding of [^{3}H]α-dihydropicrotoxinin, a γ-aminobutyric acid synaptic antagonist to rat brain membranes. Mol Pharmacol 14:391, 1978
44. Gademsetty MK: Prolonged recovery from propofol and diazepam (letter). Anaesthesia 43:611, 1988
45. Bunney BS, Sesack SR, Silva NL: Midbrain dopaminergic systems: neurophysiology and electrophysiological pharmacology, Psychopharmacology: The Third Generation of Progress, Edited by Meltzer MY. New York, Raven Press, 1987, pp 113–126