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Activation of rat locus coeruleus neuron $GABA_A$ receptors by propofol and its potentiation by pentobarbital or alphaxalone

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Abstract

The action of propofol on the rat locus coeruleus was examined using intracellular recording from in vitro brain slice preparations. Concentrations of propofol between 3 and 300 μM were tested. At 100 μM, propofol completely inhibited the firing of all neurons tested ($n = 34$); this was associated with a 5.7-mV hyperpolarization (range 0–16 mV, $n = 33$) and a 35.6% reduction in input resistance (range 7.3–66.1%, $n = 33$). The propofol-induced responses were not affected by 2-hydroxysaclofen (50 μM) or $BaCl_2$ (300 μM), but were completely blocked by bicuculline methiodide (100 μM) or picrotoxin (100 μM), indicating that propofol acts on $GABA_A$ receptors. As assessed by inhibition of the spontaneous firing rate, propofol was 5.6-fold more potent than GABA (γ-aminobutyric acid). Potentiation of the propofol effect by other general anesthetics or other drugs was also investigated. When pentobarbital (100 μM) was tested alone on locus coeruleus cells, no change in membrane potential or input resistance was seen and there was only a $20.3 \pm 7.2\%$ ($n = 8$) inhibition of firing rate; however, in combination with 30 μM propofol, it caused a 6.1-fold greater increase in membrane hyperpolarization and a 9.7-fold greater reduction in input resistance than 30 μM propofol alone. A relatively low concentration of alphaxalone (10 μM), when tested alone, had little effect on the membrane potential or input resistance and only produced a $46.0 \pm 8.9\%$ ($n = 8$) inhibition of firing rate; however, in combination with 30 μM propofol, it caused a 9.3-fold greater hyperpolarization and an 8.6-fold greater reduction in input resistance compared with 30 μM propofol alone. In contrast, diazepam caused no potentiation of either propofol- or GABA-induced responses. Our data also indicate that locus coeruleus neuron $GABA_A$ receptors possess distinctive pharmacologic characteristics, such as blocking of the propofol effects by zinc and insensitivity to diazepam and the direct action of pentobarbital. On the basis of these pharmacologic properties, we suggest that locus coeruleus neuron $GABA_A$ receptors do not contain the γ subunit.

Keywords: $GABA_A$ receptor; Anesthetic; Propofol; Locus coeruleus

1. Introduction

Propofol (2,6-diisopropylphenol) is an intravenous general anesthetic and hypnotic that is structurally unrelated to other anesthetics. In recent years, propofol has been used extensively in clinical anesthesia because of its clinical benefits of a rapid onset, clear emergence, and lack of cumulative effects (for reviews, see Fulton and Sorkin, 1995; Tagliente, 1997). Although considerable information is available on the pharmacokinetic and pharmacodynamic properties of propofol (e.g., Langley and Heel, 1988; Dundee and Clarke, 1989), its cellular mechanism of action on the central nervous system has not yet been entirely elucidated. Propofol is reported not only to potentiate γ-aminobutyric acid (GABA)-mediated inhibitory synaptic transmission (Collins, 1988; Albertson et al., 1991), but also to directly activate the $GABA_A$ receptor (Peduto et al., 1991; Hara et al., 1993). However, as far as anesthesia is concerned, GABA is not suitable for clinical use because it cannot cross the blood-brain barrier. The $GABA_A$ receptor–chloride ionophore complex is believed to be a multisubunit protein that contains binding sites for a variety of compounds. For example, GABA, barbiturates, steroids, and benzodiazepines have been suggested to bind to the $GABA_A$ receptor complex and enhance the inhibitory $GABA_A$ response via allosteric modulation of receptor function (Olsen and Tobin, 1990). The efficacy and potency of these drugs as GABA potentiators (Shingai

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et al., 1991; Horne et al., 1993) or as direct $GABA_A$ receptor activators (Valeyev et al., 1993; Adodra and Hales, 1995) may be subunit-dependent. For example, it has been shown that the potentiation of GABA responses by benzodiazepines depends on the presence of a γ subunit (Pritchett et al., 1989; Ymer et al., 1990; Knoflach et al., 1991). The aims of the present investigation were therefore to provide information on (1) the cellular mechanism of action of propofol on locus coeruleus neurons, (2) the possible correlation of the in vitro dose–response results for propofol with its clinically relevant concentrations, (3) the potency of propofol compared with GABA, (4) potentiating effects of pentobarbital, alphaxalone (a steroid type of general anesthetic), or diazepam on the propofol-evoked responses, and (5) whether locus coeruleus neuron $GABA_A$ receptors contain a γ subunit.

The locus coeruleus brain slice preparation was used in the present investigation for a number of reasons. Firstly, the locus coeruleus contains the greatest concentration of noradrenergic cell bodies in the central nervous system, projections from this small pontine nucleus being responsible for more than half of the noradrenaline terminals in the brain (Amaral and Sinnamon, 1977). Since locus coeruleus neurons have extensive projections to many central nervous system areas, changes in the activity of these neurons would be expected to cause widespread effects. Secondly, using autoradiography, GABA receptors have been demonstrated to be present in the locus coeruleus (Palacios et al., 1981) and binding studies have shown the presence of both $GABA_A$ and $GABA_B$ receptors in the terminal fields of locus coeruleus neurons (Suzdark and Gianutsos, 1985). Neurochemical studies indicate that almost half of the afferent terminals in the locus coeruleus can take up radiolabeled GABA (Iversen and Schon, 1973). Thus, the locus coeruleus provides an excellent site for studying the actions of GABA agonists. Thirdly, functional studies have demonstrated that the locus coeruleus is one of the major neural sites involved in sedation (Correa-Sales et al., 1992; Pertovaara et al., 1994) and, more importantly, it is the primary origin of descending noradrenergic analgesic fibers. Electrophysiological studies have provided strong evidence for a role of the locus coeruleus in modulating analgesia (for reviews, see Proudfit, 1988; Jones, 1991; Lipp, 1991).

2. Materials and methods

2.1. Preparation and maintenance of locus coeruleus slices

The methods used to prepare and maintain rat locus coeruleus slices were similar to those described previously (Chiu et al., 1990, 1993, 1995; Yang et al., 1998). Male Sprague–Dawley rats (120–200 g) were sacrificed and their brains rapidly removed. A block of tissue containing the pons was excised and attached to a small Plexiglass stage with cyanoacrylate glue; an agar block, next to the tissue, served to support it during sectioning. The tissue was then submerged in oxygenated artificial cerebrospinal fluid (aCSF; in mM: NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 26.2, $MgCl_2$ 1.3, $CaCl_2$ 2.4, glucose 11.1), maintained at 3–5°C, in the well of a Lancer 1000 vibratome. Coronal sections (300–400 μm thick) of the pons were cut, and a slice containing a cross-section through the caudal end of the locus coeruleus mounted in the recording chamber and allowed to equilibrate for 1 h. The slice was then completely submerged in heated (33–34°C) aCSF and the solution gassed with 95% O_2 / 5% CO_2, at a flow rate of 2.3 ml/min. In the transilluminated slice, the locus coeruleus is seen as a translucent area lying lateral to the periventricular gray, below the fourth ventricle.

2.2. Intracellular recording

Intracellular recordings were made from locus coeruleus neurons using sharp microelectrodes filled with 2 M potassium acetate with a d.c. tip resistance of 60–120 MΩ. The recording microelectrodes were inserted into the locus coeruleus under visual control. Intracellular potentials were recorded using an amplifier with an active bridge circuit, permitting the injection of current through the recording electrode (WPI M707). Current and voltage traces were displayed on a storage oscilloscope (Textronix 5113) and a rectilinear pen recorder (Gould 2400). Input resistance was measured by passing hyperpolarizing constant pulses of sufficient duration to fully charge the membrane capacitance and reach a steady-state voltage deflection. In order to minimize possible errors from inward rectification, only weak constant current pulses were used to create a voltage deflection within 10–15 mV.

2.3. Perfusion of solutions and drugs

A valve system was employed to switch the solutions superfusing the preparation from control aCSF to aCSF containing the test drugs. Standard aCSF (see above) was used to perfuse the slices throughout most of the experiments; however, since phosphate anions cause zinc salts to precipitate, standard aCSF was replaced with a modified aCSF containing no phosphate and with a lower $NaHCO_3$ concentration (12 mM) (Wong and Prince, 1981) when zinc was used. The modified aCSF was administered for 10–30 min before addition of the zinc, and any changes seen following zinc addition were compared to this base line. The time required for test solutions to reach the chamber ranged from 25 to 35 s. The drugs and chemicals used were propofol and alphaxalone (both from RBI) and bicuculline methiodide, picrotoxin, 2-hydroxysaclofen, $BaCl_2$, $ZnCl_2$, GABA, pentobarbital, and diazepam (all from Sigma), and were administered for a sufficiently long period to attain a steady-state response (6 min or more).

2.4. Data analysis

The IC_{50} was defined as the concentration which resulted in 50% inhibition of neuronal firing and was obtained by interpolating from the data points in the dose–response curves. Numerical data were expressed as the means ± the standard error of the mean (S.E.M.). Wilcoxon signed rank (paired) or rank sum (unpaired) tests were used to analyze the differences between individual means. P values equal to, or less than, 0.05 were judged to be statistically significant.

3. Results

The present results are based on recordings made from 196 locus coeruleus neurons with stable intracellular impalement. These cells had a resting membrane potential of -54.1 ± 0.4 mV ($n = 196$) and an apparent input resistance of 187.2 ± 8.2 MΩ ($n = 190$). They fired spontaneously from 0.5 to 3.8 Hz (2.1 ± 0.1 Hz, $n = 196$), with a regular interspike interval characteristic of locus coeruleus neurons in slice preparations. These data are similar to those obtained in our previous studies (Chiu et al., 1990, 1993, 1995; Yang et al., 1998).

3.1. Propofol responses

Propofol (3–30 μM) reversibly decreased the firing rate of all locus coeruleus neurons tested. However, at higher concentrations (100 and 300 μM), the effect of propofol was only partially reversible on washing the tissue with drug-free solution for periods of up to 1.5 h. Propofol (100–300 μM) was therefore applied cumulatively to each neuron. While the neurons varied in their sensitivity to propofol, all showed concentration-dependent inhibition of firing rate, hyperpolarization of membrane potential, and reduction in input resistance (Fig. 1). The decrease in firing rate was most sensitive, showing a marked change even at the lower propofol concentrations, with 3, 10, and 30 μM propofol, respectively, causing decreases of 16% ($n = 18$), 35% ($n = 31$), and 56% ($n = 41$, including nine cells in which firing was completely suppressed), whereas, at these concentrations, virtually no hyperpolarization and only a small reduction in input resistance were seen. Higher concentrations of propofol (100 and 300 μM) resulted not only in a greater inhibition of firing, but also in membrane hyperpolarization and a decreased input resistance. Considerable variation was seen in the extent of hyperpolarization and reduction of input resistance induced by the same concentration of propofol in different neurons. For example, the hyperpolarization and input resistance reduction in response to 100 μM propofol were in the range of 0–16 mV (5.7 ± 0.7 mV, $n = 33$) and 7.3–66.1% ($35.6 \pm 2.9\%$, $n = 33$), respectively. Thus, although the magnitude of the propofol-induced hyperpolarization (3.6 ± 0.8 mV, range 1.5–7 mV, $n = 8$) and input resistance decrease ($29.7 \pm 5.9\%$, range 4.8–51.5%, $n = 8$) at 300 μM was reduced compared with that at 100 μM propofol (Fig. 1), the difference was not statistically significant.

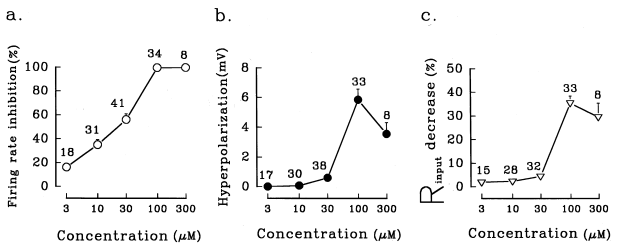

Fig. 1. Dose-dependent effects of propofol on the firing rate (a), membrane potential (b), and input resistance (c) of locus coeruleus neurons. Means ± S.E.M. are shown for the number of neurons indicated.

Table 1
The IC_{50} for GABA and propofol on the inhibition of spontaneous firing of neurons of the locus coeruleus

Cell no.	IC_{50} of GABA (μM)	IC_{50} of propofol (μM)	IC_{50} ratio (GABA/propofol)
1	199.5	33.1	6.0
2	112.2	28.8	3.9
3	288.4	38.0	7.6
4	59.6	13.5	4.4
5	33.1	5.3	6.2
Mean ± S.E.M.	138.6 ± 39.8	23.7 ± 5.5	5.6

3.2. Potency of propofol compared with GABA

Dose–response curves for propofol and GABA for the inhibition of firing rate were compared in five locus coeruleus neurons. The neurons were perfused individually with various concentrations of one of the two drugs, then with the other. A comparison of the IC_{50} for propofol and GABA in these five neurons is shown in Table 1. On average, propofol was 5.6 times more potent than GABA.

3.3. Effects of bicuculline methiodide or 2-hydroxysaclofen on the propofol-induced hyperpolarization

Perfusion with bicuculline methiodide (100 μM), a $GABA_A$ receptor antagonist, had little effect per se on locus coeruleus neuronal activity. Because the effect of propofol (100 μM) was reversed so slowly after washing, cumulative application of drugs was used. When propofol (100 μM) was first applied for 10 min to reach a steady-state response, then the perfusate changed to a combination of propofol (100 μM) and bicuculline methiodide (100 μM), the propofol-induced change in membrane potential in this neuron was reversed (Fig. 2A). Bicuculline methiodide was able to completely reverse the propofol-induced hyperpolarization in all five neurons tested, although partial suppression of the baseline firing rate was still seen ($37.9 \pm 16.7\%$, $n = 5$). In contrast, a selective $GABA_B$ receptor antagonist, 2-hydroxysaclofen (50 μM), did not antagonize the propofol (100 μM)-induced hyperpolarization of locus coeruleus neurons ($n = 3$, data not shown).

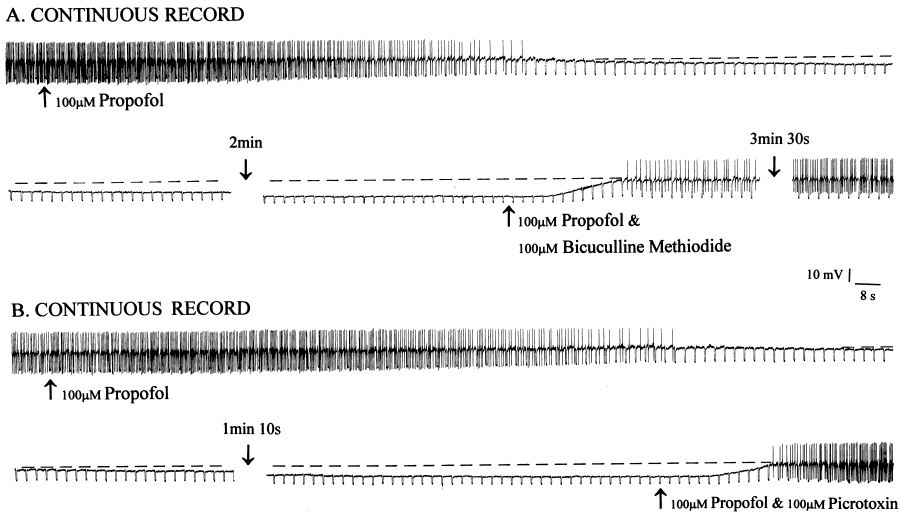

Fig. 2. Effects of bicuculline methiodide or picrotoxin on the propofol-induced hyperpolarization in a locus coeruleus neuron. (A) The cell was first pretreated with propofol (100 μM) until the maximum response was achieved (14 mV hyperpolarization and 65.9% reduction in input resistance), then the perfusate was changed to the combination of propofol (100 μM) and bicuculline methiodide (100 μM). (B) After superfusion with propofol, the same neuron was challenged with 100 μM picrotoxin, again in the presence of propofol (100 μM). Both bicuculline methiodide and picrotoxin completely reversed the propofol-induced hyperpolarization. The broken horizontal lines indicate −50 mV.

3.4. Effects of picrotoxin or $BaCl_2$ on the propofol-induced hyperpolarization

The blocking effect of picrotoxin (100 μM), a chloride channel blocker, on the propofol-induced hyperpolarization was tested on five locus coeruleus neurons. Perfusion of picrotoxin (100 μM) alone did not produce any significant changes in membrane potential or input resistance, but a small increase in firing rate was occasionally observed. When, after superfusion with propofol (100 μM) for 10 min, the same neuron was treated with a combination of propofol (100 μM) and picrotoxin (100 μM), propofol-in-

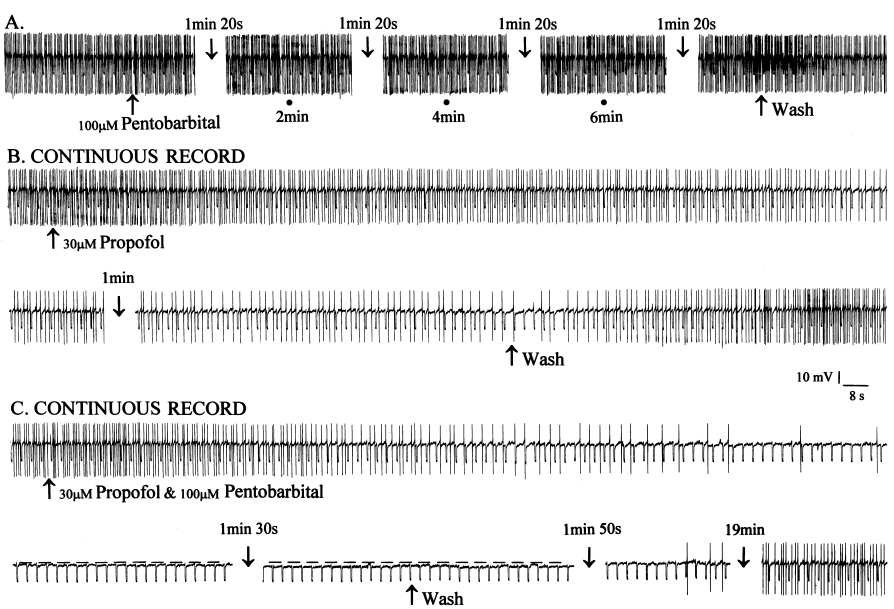

Fig. 3. Intracellular recordings from a locus coeruleus neuron illustrating the responses induced by superfusion with propofol, either alone or together with 100 μM pentobarbital. (A) Pentobarbital (100 μM) alone had no effect on the membrane potential, input resistance, and firing rate. (B) Superfusion with propofol (30 μM) caused only partial inhibition of the firing rate (47.7%) and no membrane potential hyperpolarization or reduction of input resistance. (C) In the presence of pentobarbital (100 μM), propofol (30 μM) caused complete inhibition of firing, together with hyperpolarization of the membrane potential (4 mV) and an obvious decrease in input resistance (17.6%).

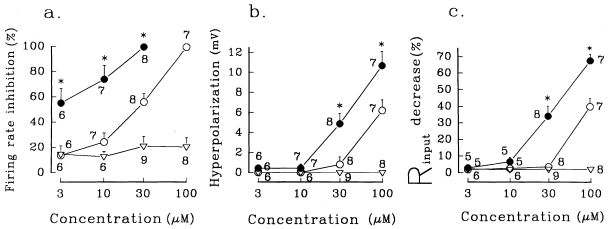

Fig. 4. Effects of pentobarbital on propofol-induced responses in locus coeruleus neurons. Firing rate inhibition (a), membrane hyperpolarization (b), and input resistance decrease (c) using propofol (○), pentobarbital (∇), or propofol plus 100 μM pentobarbital (●). The enhancement of the propofol response by 100 μM pentobarbital was significantly greater than that expected from a simple additive response, especially at the propofol concentrations of 30 or 100 μM. Note that pentobarbital alone had little effect on locus coeruleus cells, even at a concentration of 100 μM. The vertical bars represent the S.E.M. for the number of neurons indicated. $^{*}P < 0.05$, compared with the results for propofol alone using the Wilcoxon signed rank test.

duced hyperpolarization was completely reversed in all five neurons tested (Fig. 2B), although partial suppression of the baseline firing rate was still seen ($57.1 \pm 14.3\%$, $n = 5$). In contrast, barium (300 μM), a potassium channel blocker, did not block the propofol-induced hyperpolarization ($n = 3$, data not shown).

3.5. Pentobarbital potentiation of the effects of propofol

Fig. 3 shows the results from one cell, in which the effects of propofol (30 μM) were potentiated by 100 μM pentobarbital. Pentobarbital alone, even at a concentration as high as 100 μM, had no effect on input resistance, membrane potential, and firing rate (Fig. 3A); however, the same concentration of pentobarbital markedly enhanced the 30 μM propofol-induced hyperpolarization (propofol: 0 mV; propofol + pentobarbital: 4 mV), the decrease in input resistance (propofol: 2.2%; propofol + pentobarbital: 17.6%), and the inhibition of spontaneous firing (propofol: 47.7%; propofol + pentobarbital: 100%) (Fig. 3B and C). The responses using a combination of 100 μM pentobarbital and either 30 or 100 μM propofol were significantly greater than the expected additive response (Fig. 4). For example, 100 μM pentobarbital, which had no obvious effect on membrane potential or input resistance and caused only a small decrease in firing rate ($20.3 \pm 7.2\%$, $n = 8$), potentiated the effects of 30 μM propofol in terms of inhibition of firing rate, hyperpolarization, and reduction of input resistance, respectively, by a factor of 1.8-fold (propofol: 55.9%, propofol + pentobarbital: 100%, $n = 8$, Fig. 4a), 6.1-fold (propofol: 0.8 mV; propofol + pentobarbital: 4.9 mV, $n = 8$, Fig. 4b), and 9.7-fold (propofol: 3.5%, propofol + pentobarbital: 34.1%, $n = 8$, Fig. 4c). At propofol concentrations lower than 30 μM, the potentiating effect of pentobarbital was still apparent in terms of firing rate inhibition (Fig. 4a).

3.6. Alphaxalone potentiation of the effects of propofol

Alphaxalone (3–100 μM) alone produced a concentration-dependent inhibition in firing rate, hyperpolarization of membrane potential, and reduction in input resistance (Fig. 5). At a high concentration, e.g., 100 μM, alphaxalone completely inhibited the firing of all neurons tested ($n = 3$), and this was associated with a 3.8 ± 0.4 mV ($n = 3$) hyperpolarization and a $11.2 \pm 3.0\%$ ($n = 3$) reduction in input resistance. A relatively low concentration of alphaxalone (10 μM), used alone, had little effect on membrane potential or input resistance and only caused a $46.0 \pm 8.9\%$ ($n = 8$) inhibition of firing rate, but markedly increased the propofol-induced responses and significantly shifted the concentration–response curves for propofol to the left. The effects induced by the combination of propofol and alphaxalone were greater than the sum of the individual drug responses. For example, compared with 30 μM propofol alone, the combination of 30 μM propofol and 10 μM alphaxalone caused a 1.6-fold greater inhibition of firing rate (propofol: 63.2%; propofol + alphaxalone: 100%, $n = 7$, Fig. 5a), a 9.3-fold greater membrane hyperpolarization (propofol: 0.8 mV; propofol + alphaxalone: 7.4 mV, $n = 7$, Fig. 5b), and an 8.6-fold greater reduction in input resistance (propofol: 5.0%; propofol + alphaxalone: 42.9%, $n = 7$, Fig. 5c).

3.7. Interaction of diazepam with GABA and propofol

The effects of diazepam on the propofol-evoked responses were examined using the same protocol as for pentobarbital or alphaxalone. Diazepam (100 μM) had no direct action on locus coeruleus neurons and did not enhance the propofol (10–100 μM)-induced responses ($n = 3$, data not shown). The ability of diazepam to potentiate a GABA response was examined on three locus coeruleus neurons. In these experiments, when 100 μM

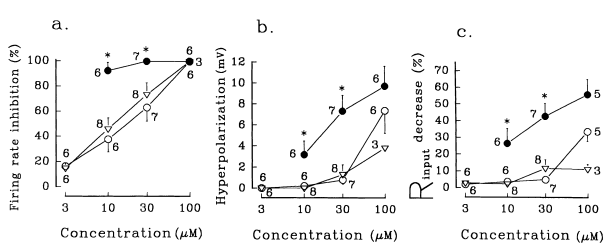

Fig. 5. Potentiation of the propofol-induced responses by alphaxalone in locus coeruleus neurons. Firing rate inhibition (a), membrane hyperpolarization (b), and input resistance decrease (c) using propofol (○), alphaxalone (∇), or propofol plus 10 μM alphaxalone (●). The enhancement of the propofol response (10–100 μM) by 10 μM alphaxalone was significantly greater than that expected from a simple additive response. The vertical bars represent the S.E.M. for the number of neurons indicated. $^{*}P < 0.05$, compared with the results for propofol alone using the Wilcoxon signed rank test.

Table 2
Blocking effects of zinc (150 μM) on propofol-induced changes in membrane potential and input resistance
Data are the mean ± S.E.M. of (n) experiments.

Condition	Hyperpolarization (mV)	Input resistance decrease (%)
Propofol (100 μM) + Zn^{2+}	0.6 ± 0.5 ($n = 5$)*	11.7 ± 3.7 ($n = 5$)*
Propofol (100 μM)	6.2 ± 1.1 ($n = 5$)	48.5 ± 4.3 ($n = 5$)

* Significantly different compared with in the absence of Zn^{2+} in the Wilcoxon signed rank test ($P < 0.05$).

GABA was applied by bath superfusion, addition of 10 μM diazepam had no effect on the GABA-induced inhibition of the firing rate (GABA: 36.8 ± 10.6%; GABA + diazepam: 31.6 ± 9.2%, $n = 3$, $P > 0.1$), decrease in input resistance (GABA: 2.9 ± 0.6%; GABA + diazepam: 2.3 ± 0.7%, $n = 3$, $P > 0.1$), or membrane hyperpolarization (GABA: 0 mV; GABA + diazepam: 0 mV, $n = 3$).

3.8. Effect of $ZnCl_2$ on the propofol-induced hyperpolarization

Perfusion of locus coeruleus neurons with zinc (150 μM) produced no significant change in membrane potential or input resistance, but caused a small increase in firing rate (59.1 ± 9.6%, $n = 5$). When, after superfusion with zinc for 15 min, the same neurons were perfused with propofol (100 μM) in the presence of zinc, the propofol-induced effects were blocked (Table 2). On average, a membrane hyperpolarization of 0.6 ± 0.5 mV ($n = 5$), a reduction in input resistance of 11.7 ± 3.7% ($n = 5$), and a partial suppression of baseline firing rate (77.6 ± 9.2%, $n = 5$, including one cell in which firing was completely inhibited) were observed. When the effects reached the steady state (about 8–10 min), propofol alone (100 μM) was then applied and produced complete inhibition of the firing of all neurons tested ($n = 5$), associated with a 6.2 ± 1.1 mV ($n = 5$) hyperpolarization and a 48.5 ± 4.3% ($n = 5$) reduction in input resistance, similar to the results for propofol treatment alone (Fig. 1), indicating that the effect of Zn^{2+} was reversible.

4. Discussion

4.1. Propofol effects on locus coeruleus activity

The present investigation demonstrates that propofol can inhibit the spontaneous firing rate, cause hyperpolarization of the membrane potential, and reduce the input resistance of locus coeruleus neurons. Of these effects, the decrease in firing rate was most sensitive, showing a marked change even at low propofol concentrations (3–30 μM). A similar result was obtained when μ-opioid receptor agonists (Williams and North, 1984; Chiu et al., 1993), α_2-adrenoceptor agonists (Chiu et al., 1995), or somatostatin receptor agonists (Chiu et al., 1994) were tested on locus coeruleus neurons. This differential sensitivity was interpreted by Williams and North (1984) in terms of a balancing effect between intrinsic membrane inward currents and μ-opioid agonist-activated outward potassium currents. This concept can also be used to explain the present propofol results as follows. The spontaneous firing of locus coeruleus neurons results from the persistent inward calcium current (Williams et al., 1984) and the cAMP-activated inward sodium current (Wang and Aghajanian, 1987); firing is inhibited when the propofol-activated outward chloride current balances the persistent inward calcium and sodium currents, while higher propofol concentrations cause a further increase in the outward chloride current, thus hyperpolarizing the cell. Furthermore, the spontaneous firing rate of locus coeruleus neurons is also influenced by two main synaptic inputs, namely the glutamatergic input from the nucleus paragigantocellularis and the GABAergic projection from the nucleus prepositus hypoglossi (Aston-Jones et al., 1991). Thus, it is possible that the presynaptic actions of propofol may also play a significant role in influencing the neuronal activities of the locus coeruleus. Recently, two independent studies in rats have demonstrated that propofol can cause presynaptic inhibition of Na^+ channel-dependent glutamate release from cerebrocortical synaptosomes (Ratnakumari and Hemmings, 1997) and inhibition of GABA uptake (accumulation of synaptic GABA) by striatal synaptosomes (Mantz et al., 1995). Thus, in addition to its direct postsynaptic effects, propofol may also indirectly inhibit locus coeruleus neuronal activity by blocking glutamate release or GABA uptake at the presynaptic site. This presynaptic action might also account for the differential sensitivities of the firing rate, input resistance and membrane potential seen in response to propofol.

4.2. Activation of the $GABA_A$ receptor–chloride ionophore complex by propofol

Propofol has been shown to affect the GABAergic system in the brain (see Introduction). However, GABA activates two distinct types of receptors (Sivilotti and Nistri, 1991), one of which, the $GABA_A$ receptor, is a ligand-gated chloride channel, while the other, the $GABA_B$ receptor, is a G protein-coupled receptor which modulates potassium channels. Electrophysiologic and binding studies indicate that both types of GABA receptor are present on locus coeruleus neurons (Suzdak and Gianutsos, 1985; Olpe et al., 1988; Shefner and Osmanovic, 1991). In the present study, the $GABA_A$ receptor antagonists, bicuculline methiodide (100 μM) and picrotoxin (100 μM), both blocked the effects of 100 μM propofol, whereas a $GABA_B$ receptor antagonist, 2-hydroxysaclofen (50 μM),

or a potassium channel blocker, barium (300 μM), had no effect. These results demonstrate that propofol exerts its effects via the $GABA_A$ receptor–chloride channel complex.

4.3. Clinical implications

Correlation of the in vitro effects of propofol with its clinically relevant concentrations is an important, but not straightforward, consideration. The estimation of appropriate concentrations of many intravenous anesthetics is complicated by their binding to proteins and by the technically difficult feat of measuring their brain concentrations during anesthesia. The blood concentrations of propofol that induce anesthesia in humans range from 17 to 35 μM (Gepts et al., 1985; Vuyk et al., 1992; Wakeling et al., 1999), while those that induce sedation range from 0.6 to 11 μM (Vuyk et al., 1992; Smith et al., 1994). Assuming that the brain/blood propofol concentration ratio is 3 (Shyr et al., 1995), then the corresponding brain concentrations of propofol for loss of consciousness or sedation would range, respectively, from 51 to 105 μM, and from 1.8 to 33 μM. The present study demonstrates that the only effect of low concentrations (3–30 μM) of propofol was a partial inhibition of the neuronal firing rate, whereas higher concentrations (> 30 μM) had more marked effects, including membrane hyperpolarization. It is interesting to note that these two concentration ranges correlate well with the clinically relevant ranges for causing sedation (1.8–33 μM) or unconsciousness (51–105 μM) in man. The present study might, therefore, provide experimental data at the single neuron level to explain the difference in individual neuronal activity of the brain between the states of sedation and unconsciousness.

4.4. Comparison of the potencies of propofol and GABA

The depressant effects of propofol on locus coeruleus neurons were more potent than those of GABA. In terms of inhibition of neuronal firing, propofol was 5.6 times more potent than GABA. This finding differs from the results of previous whole-cell patch clamp studies on cultured hippocampal (Orser et al., 1994) or hypothalamic (GT1-7) neurons (Adodra and Hales, 1995), both of which showed that propofol was less effective than GABA as a $GABA_A$ receptor activator. In this respect, it should be noted that, in the present experiments, the system for the uptake of GABA into glial and neuronal elements (for review, see Krnjević, 1984) was not inhibited, so the exact GABA concentration at the receptor sites is not known. Moreover, the extent of GABA receptor desensitization at different concentrations of GABA is unknown, although noticeable fading of GABA responses is only seen at concentrations higher than 5 mM (Osmanovic and Shefner, 1990). However, despite these possible GABA uptake and desensitization effects, the apparent difference in the sensitivity and pharmacology of $GABA_A$ receptors in different neurons may be due to the subunit composition of the receptor. For example, β_1 $GABA_A$ homomeric receptors, when expressed in *Xenopus* oocytes, show a high sensitivity to propofol, but a low sensitivity to GABA, in terms of activating chloride currents. Adding a α_1 subunit to the receptor increases the sensitivity to GABA and reverses the relative potency of propofol and GABA (Sanna et al., 1995a). The γ_2 subunit also affects the relative potency of GABA and propofol in *Xenopus* oocytes (Jones et al., 1995; Sanna et al., 1995b; Lam and Reynolds, 1998). At the present time, there is insufficient information about the subunit stoichiometry of different receptors to address the difference in drug sensitivities seen in various neuronal preparations. This higher potency of propofol compared with GABA may be an important factor in distinguishing locus coeruleus $GABA_A$ receptors from those of other central neurons.

4.5. The potentiation of propofol-induced effects by pentobarbital or alphaxalone

The present results demonstrate that the combination of propofol with either pentobarbital or alphaxalone (a steroid-type general anesthetic) produced an enhancement of the propofol response that was significantly greater than a simple additive effect. Since propofol appears to bind to a site on the $GABA_A$ receptor that is distinct from the binding site for barbiturates or alphaxalone (Cancas et al., 1991), this potentiation might be due to a conformational change induced in the receptor by pentobarbital or alphaxalone (Sieghart, 1992). Interestingly, a previous biochemical study showed that the combination of either pentobarbital or alphaxalone with propofol only had a simple additive effect on the muscimol-stimulated $^{36}Cl^-$ uptake and ^{3}H-GABA binding of cortical membrane preparations (Cancas et al., 1991). A possible explanation for the differences in the degree of propofol potentiation between our results and those of Cancas' group might be the presence of a γ-subunit in the cortical neuron $GABA_A$ receptor (McKernan and Whiting, 1996). Previous studies have shown that either pentobarbital (Study and Barker, 1981) or alphaxalone (Callachan et al., 1987) can increase the GABA-induced channel open time, and Horne et al. (1993) further suggested that this effect is reduced by the presence of the γ subunit, i.e., the γ subunit significantly reduces both the efficacy and potency of pentobarbital or alphaxalone in the potentiation of the GABA response. In contrast to cortical neurons, locus coeruleus neuron $GABA_A$ receptors may not possess a γ subunit (see the next paragraph), and this may explain the observed synergistic potentiation.

4.6. The $GABA_A$ receptors of the locus coeruleus neuron do not contain the γ subunit

Although the subunit composition of the $GABA_A$ receptor in locus coeruleus neurons is still unknown, on the basis of an immunohistochemical study, γ_2 subunits have been claimed to be present (Fritschy et al., 1992). Other groups, however, have been unable to detect γ_2 subunit mRNAs in the locus coeruleus (Araki et al., 1992a,b; Luque et al., 1994; Tohyama and Oyamada, 1994), but did find weak expression of γ_1 subunit mRNAs. Although the source of this disparity remains unclear, mRNA levels do not always reflect protein levels. In the present study, we characterized the γ subunit of $GABA_A$ receptors in locus coeruleus neurons by pharmacologic means. The results suggest that locus coeruleus neuron $GABA_A$ receptors probably do not contain the γ subunit. This conclusion is based on three pieces of experimental evidence. Firstly, in locus coeruleus neurons, diazepam was unable either to directly activate the $GABA_A$ receptor or enhance GABA- or propofol-induced responses. Since several lines of evidence indicate that a benzodiazepine binding site cannot be formed in the absence of a γ subunit (Pritchett et al., 1989; Whiting et al., 1995) and that $GABA_A$ receptors lacking the γ_1, γ_2, or γ_3 subunit are insensitive to benzodiazepines (Pritchett et al., 1989; Ymer et al., 1990; Knoflach et al., 1991), the most probable explanation would be that locus coeruleus neuron $GABA_A$ receptors lack the γ subunit. Secondly, it is well documented that zinc only blocks $GABA_A$ receptor complexes that lack a γ subunit (Draguhn et al., 1990; Smart et al., 1991). However, in the present study, the propofol-evoked responses were significantly blocked by zinc, therefore, it is reasonable to suggest that the $GABA_A$ receptor complex in rat locus coeruleus neurons does not have a γ subunit. Thirdly, Valeyev et al. (1993) reported that pentobarbital (100 μM) did not activate rat brain $\alpha_1\beta_2$ and $\alpha_1\beta_3$ $GABA_A$ receptors expressed in cultured Chinese hamster ovary cells; however, these same receptor combinations were activated by GABA, and the GABA-evoked currents were markedly potentiated by barbiturate. In contrast, pentobarbital is able to activate $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors expressed in oocytes (Amin and Weiss, 1993). On the basis of the pentobarbital data from the two above laboratories and their own, Jones et al. (1995) reached the conclusion that direct pentobarbital activation of the $GABA_A$ receptor requires the γ subunit. In the present study, pentobarbital alone had no effect on either membrane potential or input resistance when applied at a high concentration (100 μM) (see also Osmanovic and Shefner, 1990). Taken together, these experimental results argue against the presence of the γ subunit in the locus coeruleus neuron $GABA_A$ receptor.

4.7. Summary

We have shown that, in locus coeruleus neurons in vitro, propofol causes the opening of $GABA_A$-gated chloride channels, resulting in membrane hyperpolarization at clinically relevant concentrations, with a potency six-fold greater than that of GABA. Our data also demonstrate that locus coeruleus neuron $GABA_A$ receptors have distinctive pharmacologic characteristics, such as a synergistic, rather than additive, potentiation of the effects of propofol by pentobarbital or alphaxalone, the blocking of the effects of propofol by zinc, and an insensitivity to diazepam and the direct action of pentobarbital. Based on these pharmacologic properties, we suggest that locus coeruleus neuron $GABA_A$ receptors do not contain the γ subunit.

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