



PII S0091-3057(96)00299-7

Responses to Propofol in Relation to GABA Functionality of Discrete Parts of the Brain of Rats

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Received 18 January 1996; Revised 25 May 1996; Accepted 17 July 1996

DIRKSEN, R., B. ELLENBROEK, J. VAN EGMOND, AND A. R. COOLS. *Responses to propofol in relation to GABA functionality of discrete parts of the brain of rats.* PHARMACOL BIOCHEM BEHAV 57(4) 727–735, 1997.— Genetically-determined regional differences in the GABA-ergic make-up of the brain exist in two lines of Wistar rats viz.: apomorphine-susceptible (APO-SUS) and apomorphine-unsusceptible (APO-UNSUS) Wistar rats. Propofol is a GABA-mimetic general anesthetic. This study compared the responses to propofol in APO-SUS and APO-UNSUS rats. Propofol induced a higher incidence of involuntary muscular contractions and oral movements, but a lower incidence of grooming, in APO-SUS rats than in APO-UNSUS rats. Reflex inhibition and narcosis, being defined as the behavior marked by both full absence of purposeful movements and by complete loss of righting reflexes, after propofol did not differ between the two lines. APO-SUS rats had less variation of the heart rates and greater variations of diastolic arterial pressures in response to electrical stimulation than the APO-UNSUS rats, and these variations were reduced by increasing doses of propofol. Arterial pressures in APO-SUS rats were higher than in APO-UNSUS rats. Propofol caused a biphasic change in intra-arterial pressures and had the greatest effect in APO-SUS rats. Differences in cerebral GABA transmission, especially in the striato-nigro-collicular pathway, did not give rise to differences in the effect of propofol on narcosis and hindlimb withdrawal reflex. In contrast, these differences in GABA transmission were accompanied by line-specific differences in effect of propofol on certain behavioral and cardiovascular parameters. © 1997 Elsevier Science Inc.

General anesthetic Intravenous anesthetic Propofol anesthetic Individual rat variation
Apomorphine-susceptible rat Apomorphine-unsusceptible rat Behavioral response Cardiovascular response

GENERAL anesthesia involves narcosis, reflex suppression and relaxation (19). GABA neurotransmission plays a role in anesthetic-induced narcosis (18,29,30,31,38,39) and associated changes in cognition (vigilance), behavior, motor performance, reflexes and cardiovascular regulation (7,25,27,39). Recently, it was found that inbred differences in response to diazepam, a drug that can indirectly enhance GABA transmission in the brain, are associated with differences in volatile anesthetic requirements (28). This suggests that the response to anesthetics which affect the GABA transmission, may vary according to genetically determined differences in the GABA-ergic make-up of the brain. In the present study, we investigated to what extent the effects of propofol, an anesthetic that among others enhances the GABA function (1,9,21,32),

vary in rats which have determined differences among others in the GABA-ergic make-up of the brain (10,11,12,33).

The apomorphine-susceptible (APO-SUS) and the apomorphine-unsusceptible (APO-UNSUS) Wistar rat lines were chosen. These rat lines are marked by a mirror image of the functional activity of GABA transmission in the striato-nigro-collicular pathway: APO-SUS rats have a relatively low activity at the level of the substantia nigra, pars reticulata and a relatively high activity at the level of the deeper layers of the colliculus superior. APO-UNSUS rats show precisely the opposite activity in these brain regions (10). Propofol [2,6-di-isopropyl phenol (Diprivan®)] was chosen as the anesthetic because at least part of its actions is due to its ability to enhance the function of the GABA_A/ionophore receptor com-

plex (1,9,21,32), and it is noteworthy that propofol shares the ability to take effect via this site with various chemically distinct classes of general anesthetics agents (38). An additional criterion for selecting propofol is its short duration of effect, especially as a pilot study showed that this feature allowed for the assessment of dose response curves in individual rats. Four types of the main responses to propofol, i.e. narcosis, inhibition of reflexes, drug-evoked behavioral signs, and cardiovascular changes, were studied (3,4,6,8,24,26,34).

METHOD

Animals

Subjects were nine female apomorphine-susceptible (APO-SUS; body weight 255 ± 2 g) and nine female apomorphine-unsusceptible (APO-UNSUS; body weight 225 ± 6 g) Wistar rats bred and reared in the Central Animal Laboratory, University of Nijmegen, the Netherlands. Rats belonging to the 13th generation of the APO-SUS and APO-UNSUS lines were used. Parents of the APO-SUS and APO-UNSUS lines, which had produced 100% offspring with apomorphine-induced gnawing scores characteristic for their line, were identified. Only naive female rats, belonging to the second litter of these parents, were used in the present study. Further procedural details on animal breeding, maintenance and selection of these rat lines have been described elsewhere in detail (10). The rats were housed 2–3 animals per Macrolon cage ($36 \times 24 \times 25$ cm) and received manufactured food and tap water ad lib. They were kept at a 12 h light/dark cycle with white lights on at 0600 A.M., and the environmental temperature was 20°C (range 18 – 22°C).

Drug

Propofol (2,6-di-isopropyl phenol (Diprivan®)) dissolved in its vehicle (aqueous emulsion containing 10% w/v Soya bean oil, 1.2% w/v egg phosphatide and 2.25% w/v glycerol) was intravenously (IV) given in a volume of 1 ml.kg^{-1} . The doses of 20 or 40 mg.kg^{-1} were delivered in a volume of 2 and 4 ml.kg^{-1} , respectively.

PREPARATIONS

Study on Narcosis and Drug-Induced Behavior

A cannula (Insyte® 24 G, Becton Dickinson, USA) was introduced into a tail vein and connected via an extension set with T (venisystems™, ABBOTT, Belgium) to a 1 ml syringe (Monoject Tuberculin, Sherwood Medical, Crawley, Sussex, UK). Once secured, the injection of propofol was given, and the rat was placed on a flat, wooden table ($60 \times 80 \times 90$ cm). The behavior was analyzed until the rat was fully recovered from the drugs effect (see below).

Study on Withdrawal Reflex and Cardiovascular Variables

The rat was anesthetized with an intraperitoneal (IP) injection of urethane (1.2 g.kg^{-1}) to allow cannulation of the right internal jugular vein. When the jugular vein was cannulated, aliquots urethane were injected intravenously to a total of 0.2 g.kg^{-1} , and the trachea and right carotid artery were cannulated. This total dose of urethane (1.4 g.kg^{-1}) delivered within the first half h maintained anesthesia during the experiments. After cannulation, the rat was placed on the experimentation table. The trachea cannula was used for artificial normoventilation. The cannula in the carotid artery was connected to a

pressure transducer (Viggo-Spectramed, BOC, USA) which provided a measure for the intra-arterial (IA) pressures. The heart rate was derived from the arterial pressure signal (heart rate counter AT-601G, Nihon Kohden Corp., Japan). The right hind paw was mounted in a shoe which contained two electrodes allowing transcutaneous bipolar stimulation (Grass stimulator S11, with stimulus isolation unit SIU5A, and constant current unit CCU1A). Stimulation parameters were set to 4 ms pulse duration, 7.5 mA stimulus strength, 100 Hz pulse frequency, in a train of 500 ms duration, and a repetition rate of 12.5 mHz (0.75 min^{-1}) for the trains (i.e. every 80 s a stimulus). The hind paw was also connected to a force-displacement transducer (TB-611T, Nihon Kohden Corp., Japan), which allowed the measurement of the withdrawal force response to the electrical stimulus. Details on the method have been described elsewhere (13).

EXPERIMENTAL DESIGN AND MEASURES

Study on Narcosis and Drug-Induced Behavior

Different doses of propofol had been tested in a pilot study using outbred Wistar rats. Attention was paid to (a) occurrence of narcosis (criterion for start of narcosis is abolishment of purposeful movements, at which moment the ability to regain upright position is lost. The endpoint of effect was the moment of head lift), (b) absence of toxic effects (e.g. cardiovascular collapse), (c) behavioral symptoms preceding and/or following narcosis, (d) presence/absence of tolerance, and (e) that propofol's vehicle was without effect.

On the basis of the outcome of that study, we selected the solvent of propofol (see Drug) and chose the following dosing schemes. Propofol was administered in the doses of 4, 10 and 20 mg.kg^{-1} , IV. Each dose was injected several times per trial: $n = 4/4 \text{ mg.kg}^{-1}$, IV; $n = 3/10 \text{ mg.kg}^{-1}$, IV; $n = 2/20 \text{ mg.kg}^{-1}$, IV. The interinjection interval was determined per individual and terminated as soon as the rat lifted its head. The total number of trials was 3 per animal; with an interval of 1 week between trials; there were 9 rats in each group. Each class of rat (APO-SUS and APO-UNSUS) was divided in three subgroups. The first group received 4 consecutive injections of 4 mg.kg^{-1} in week 1, 3 consecutive injections of 10 mg.kg^{-1} in week 2, and 2 consecutive injections of 20 mg.kg^{-1} in week 3. The second and third groups received the same treatments, but in another order: 20 mg.kg^{-1} , 10 mg.kg^{-1} and 4 mg.kg^{-1} (second group), and 10 mg.kg^{-1} , 20 mg.kg^{-1} and 4 mg.kg^{-1} (third group). Since the pilot study had shown that the effect of the first injection often differed from that of the consecutive injections, these effects were separately analyzed.

The pilot study was also used for selecting the behavioral items to be analyzed: narcosis, blinking with the eyes, involuntary muscular contractions (IVMC), grooming, vacuous chewing with tongue protrusions, ear retraction, head and wet dog shakes, cyanosis, excitation (escape efforts, wild gross movements), apnea, tail flicking (TF) and perturbed coordination. Apart from measuring the duration of narcosis in minutes (min), the incidence of all remaining items was scored and the incidence was expressed as percentage per injection.

Study on Withdrawal Reflex and Cardiovascular Variables

One week after the assessment of the behavioral effects, the rats were anesthetized with urethane. Determination of drug effects started 1 h after termination of the surgical preparation. In earlier studies, we had found that there are no significant fluctuations after that interval (13). The individual

base-line of withdrawal force (F , expressed in g), intra-arterial (IA) pressures (mmHg) and heart rate (bpm) were then measured for 20 min. Consecutive IV injections of propofol through the jugular catheter formed the array of 0.75, 1.25, 2.5, 5, 10 or 40 $\text{mg}\cdot\text{kg}^{-1}$ of propofol, thereby including subanesthetic and anesthetic doses, and the midst of two stimuli (40 s after an transcutaneous electrical stimulus) was the moment of injection. Consecutive injections were given once the force of the withdrawal responses was returned to pre-injection strength (see Figure 1: tension). For, the mentioned pilot study had shown that the propofol-induced change in the withdrawal reflex is the last symptom seen in rats which fully recover from propofol (13,14,15).

Data were sampled at 200 Hz (PC486–66Mhz; 8 Mb; fitted with a DAS-16 A/D board (Keithley Instr. Incorp. USA) with 48,000 samples for each 80 s period (the stimulus interval). The measures for withdrawal reflex activity were: the magnitude of force of withdrawal (g) and indices for contraction [the bl^{peak} ($\Delta\text{force}/\Delta t$), with Δt = interval (min) between stimulus and maximum withdrawal], and for relaxation [the peak^{bl} ($\Delta\text{force}/\Delta t$), with Δt = interval (min) between max. withdrawal and return to resting value]. The time course of reflex suppressive effect was assessed in terms of time constants (τ ; min) of onset (τ_o) and decay (τ_d) of the effect on the force of withdrawal. The measures for cardiovascular variables were: changes in heart rates (bpm) and changes in IA systolic (SAP) and diastolic (DAP) pressures (mmHg). Variation of these variables was assessed within the stimulus interval:

$$\int_0^{80} |\Delta\text{variable}|(t)$$

Figure 1a shows both the cardiovascular response and the withdrawal reflex in a naive rat: it illustrates the indices used in data analysis. In Figure 1b, the effect of 10 $\text{mg}\cdot\text{kg}^{-1}$ propofol in the same rat is shown to illustrate how the effects of propofol on these indices were analyzed.

STATISTICAL ANALYSIS

Statistical data handling was performed using SAS (SAS-PC v. 6.03, SAS Institute Inc., Cary NC). Two-tail probabilities were used in all tests; the level of statistical significance was set as $p \leq 0.05$.

Study on Narcosis and Drug-Induced Behavior

The duration of narcosis was analyzed using ANOVA. Line differences in the incidence of IVMCs, vacuous chewing and grooming were assessed with the help of the Fisher exact test. Effect of dose was assessed with the help of $k \times 2$ tables, with comparison of the effect among two doses assessed with 2×2 tables.

Study on Withdrawal Reflex and Cardiovascular Variables

The effects were analyzed using ANOVA, with covariance analysis (indicated by DF (1)) or classes for dose. Where appropriate ANOVA was supplemented with Scheffé's method for multiple comparisons. Data points are given as means and SEMs. Analysis of variables was performed on the values (in units of measurement) and on the change of these variables in terms of percentage of the mean of the individual base line before drug and the minimum response after injection of a dose of the drug.

The reflex suppressive effects were compared using the equation of the sigmoid Emax model (22,23). Fitting this equa-

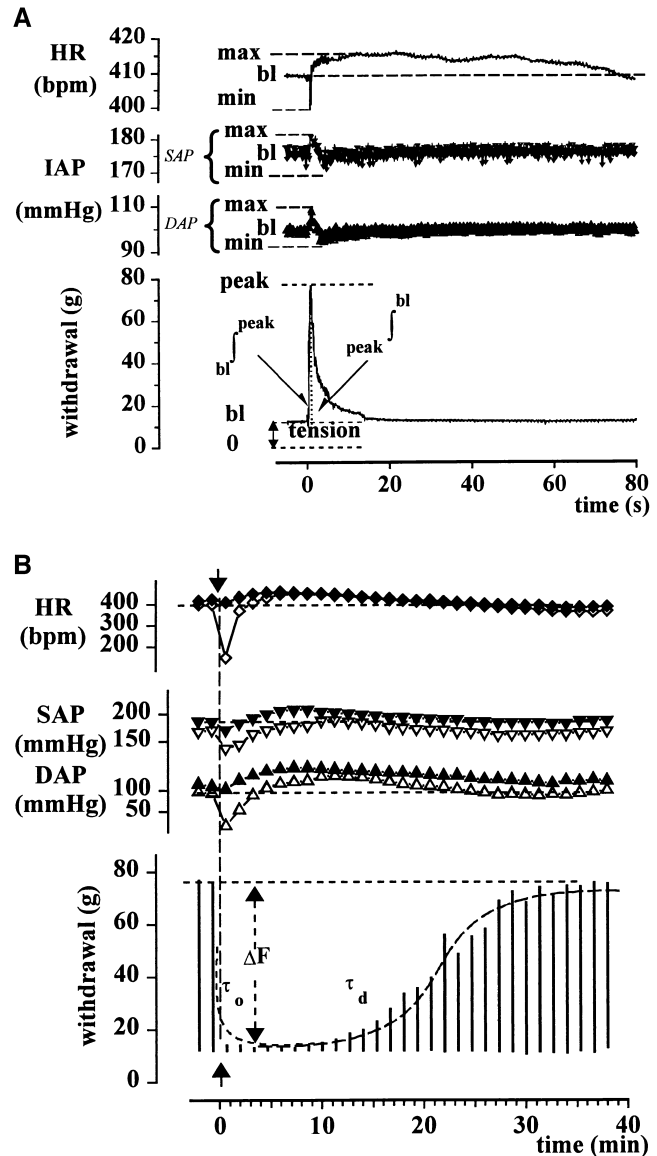


FIG. 1. Responses as a function of time of APO-SUS rat n° 2 at two time scales (s and min), and the measures for effect are indicated: 1A) the responses of heart rate (HR), intra-arterial pressures (IAP; with SAP = systolic intra-arterial pressure; and, DAP = diastolic intra-arterial pressure), and reflex activities (withdrawal) of the urethane anesthetized rats to the standard transcutaneous stimulus. This electrical stimulus is given at 0 seconds. Max, bl, and min mean the highest, the base line, and the lowest values of a variable in the 80 s period, respectively. The change of force (peak minus tension; g) and integrals for withdrawal (bl-peak) and relaxation (peak-bl) were calculated for each reflex. 1B) Pharmacological response of variables to 10 $\text{mg}\cdot\text{kg}^{-1}$ of propofol. The injection is given at 0 min (arrow). The filled and open symbols represent the highest (max in Figure 1a) and lowest (min in Figure 1a) values of HR, SAP, and DAP, respectively. The lower part of the figure shows the change of force of withdrawal (ΔF), with onset (τ_o) and decay (τ_d) of this response.

tion to the data yielded three parameter estimates: E_{max} = maximum effect, γ = exponent (Hill coefficient; slope), and ED_{50} = dose causing 50% of the effect. Using these estimates, the kinetics of the pharmacological response were analyzed

TABLE 1
THE INCIDENCE (%) AND DURATION (MIN) OF NARCOSIS
IN RELATION TO THE DOSE (mg.kg⁻¹) OF PROPOFOL

Dose	Injection	APO-SUS		APO-UNSUS	
		Incidence	Duration (mean ± SE)	Incidence	Duration (mean ± SE)
4	first	0	0	22	0.7 ± 0.5
	consecutive	88	5.7 ± 0.7	100	7.3 ± 0.4
10	first	77	7.8 ± 1.5	100	9.7 ± 0.7
	consecutive	100	13.3 ± 0.3	100	11.9 ± 0.6
20	first	100	16.2 ± 0.9	100	17.7 ± 0.4
	consecutive	100	18.0 ± 0.9	100	22.3 ± 1.3

APO-SUS = apomorphine-susceptible rats; APO-UNSUS = apomorphine-unsusceptible rats.

according to a one compartment open model (40). The mathematical model included the full dose range and yielded the estimates of the time constants of onset and decay of effect of each individual rat.

RESULTS

Narcosis in APO-SUS and APO-UNSUS Rats

The incidence and duration of narcosis of APO-SUS and APO-UNSUS rats are given in Table 1. There was no significant difference in the incidence of propofol-induced narcosis, not even when the effects of the first injections and the consecutive injections were separately analyzed.

The duration of narcosis depended on the dose of propofol and lasted longer after repeated injections, with no difference for APO-SUS and APO-UNSUS rats as indicated by the outcome of ANOVA with dose, repetition, line and body weight as factors [dose: $F(2, 153) = 273.04, p < 0.0001$; repetition: $F(3, 153) = 30.77, p < 0.0001$; line: $F(1, 153) = 2.64, p = 0.1$; body weight: $F(1, 153) = 1.70, p = 0.2$].

Propofol-Induced Behavior in APO-SUS and APO-UNSUS Rats

Propofol resulted in a significant line-difference in involuntary muscular contractions (IVMCs), vacuous chewing with tongue protrusion and grooming (Figure 2).

The difference for the incidence of IVMCs in the two lines was remarkable ($p < 0.0001$) and the incidence related to the dose [$\chi^2 (17.5), p < 0.0001$]. When treated with 10 or 20 mg.kg⁻¹ propofol: APO-SUS rats had an incidence of 90–100% IVMCs, whereas APO-UNSUS rats had only an incidence of 30–40% IVMCs (4 mg.kg⁻¹ different from 10 and 20 mg.kg⁻¹: $p = 0.004$ and 0.001 , respectively). The incidence of IVMCs was similar for first and consecutive injections ($p = 1.0$).

Vacuous chewing with tongue protrusion had a significantly higher incidence in APO-SUS than in the APO-UNSUS rats ($p = 0.037$); the incidence related to the dose [$\chi^2 (28.29), p < 0.001$; post hoc: 4 mg.kg⁻¹ different from 10 and 20 mg.kg⁻¹; $p < 0.001$ and < 0.001 , respectively]. No difference was found for the incidence of this behavior after first or consecutive injections ($p = 0.7$).

The incidence of grooming was lower in the APO-SUS than in the APO-UNSUS rats (24% versus 39%, $p = 0.01$), and the difference was especially evident after the consecutive injections (incidence after first versus consecutive injections:

7% and 46%, respectively; $p < 0.001$). There was no relationship of effect and dose [$\chi^2 (3.8), p = 0.1$].

There was no significant line-specific difference in the incidence of eye lashing (54% and 63%), ear retraction (25% and 31%), wet dog shake (20% and 15%), cyanosis (12% and 21%), excitation (12% and 9%), apnea (6% and 7%), tail flick (5% and 1%), or disturbance of coordination (4% and 0%). However, this study used a limited number of rats and additional differences may prove to be present when a larger population of rats is subjected to testing.

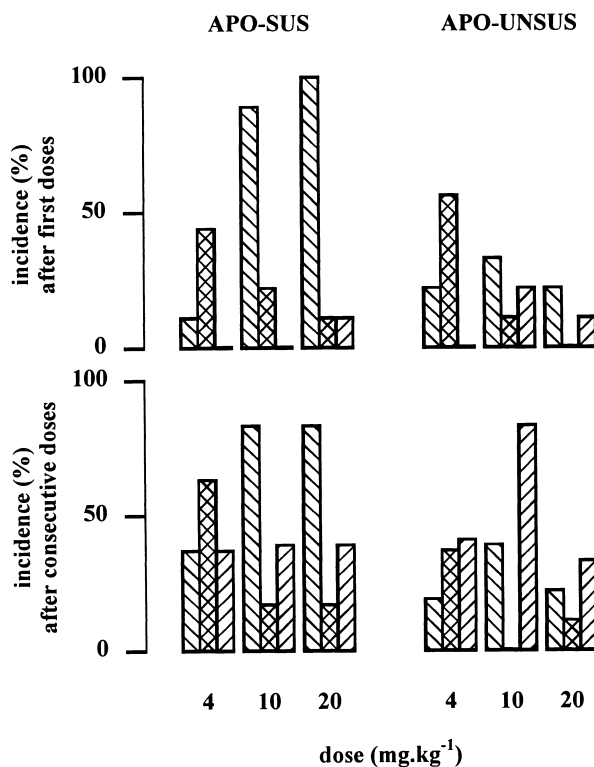


FIG. 2. Incidences (%) of propofol-induced behavior that differed for APO-SUS and APO-UNSUS rats, and the bars represent: ▨: involuntary muscular contractions, ▩: vacuous chewing with tongue protrusion, ▧: grooming.

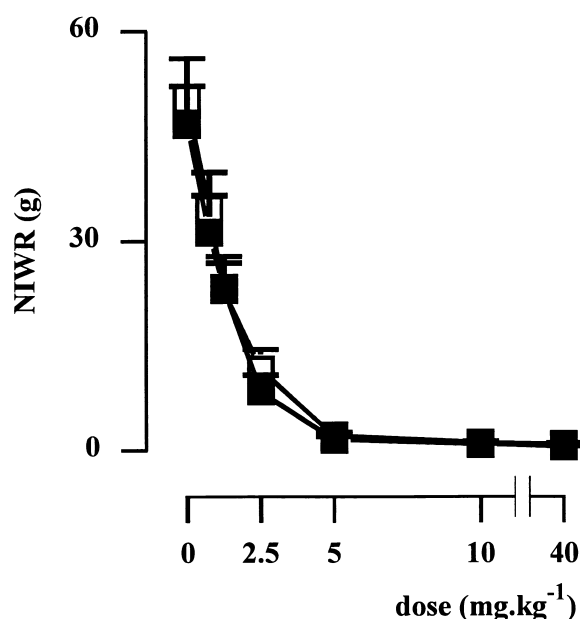


FIG. 3. The magnitude of the withdrawal response (mean; gram) in relationship to the dose of propofol ($\text{mg}\cdot\text{kg}^{-1}$) (□: open = APO-SUS rats; filled = APO-UNUSUS rats; the bars represent the SEM).

Propofol-Induced Changes in Withdrawal Reflex of APO-SUS and APO-UNUSUS Rats

The pre-drug values of withdrawal reflexes did not differ between the two lines of rats. Propofol reduced the force of withdrawal response in a dose-dependent fashion, and this effect did not differ between the two lines of rats [two-way ANOVA, dose: $F(1, 115) = 32.31, p < 0.0001$; line $F(1, 115) = 0.3, p = 0.6$; Fig. 3].

Likewise, the percentage change of contraction was not different between the two lines [dose: $F(1, 115) = 41.33, p < 0.0001$; line $F(1, 115) = 0.00, p = 0.96$]. Table 2 lists the means of the parameter estimates and standard errors of the estimates for γ and ED_{50} after fitting the equation to the data on the change of withdrawal in terms of percentage for each individual rat (marked differences were found for parameter estimates in the asymptotic correlation matrices); E_{max} maximized at 100%.

Although there was a slight but significant difference of the τ_{decay} of the pharmacological response between APO-SUS rats and APO-UNUSUS rats (Table 2; Figure 1), there was no significant effect of line and body weight.

The duration of actual contraction and that of subsequent relaxation of the hind limb were diminished in a dose-depen-

dent fashion, and these effects, or the change in terms of percentage did not differ between APO-SUS and APO-UNUSUS rats (data not shown). After the return of the force of withdrawal to pre-propofol levels, $\text{peak}^{\text{bl}}(\Delta\text{force}/\Delta t)$ was enhanced in a dose related fashion, $F(1, 115) = 5.46, p = 0.0001$, and this effect was not different between the two lines.

Cardiovascular Variables: Heart Rate and Intra-Arterial Pressures

The base-line levels of the heart rates did not differ between the two lines of rats under urethane anesthesia, but the variation of the heart rate ($_{\text{0}}^{\text{HR}}$) was significantly less for APO-SUS rats ($230 \pm 55 \text{ bpm}\cdot 80 \text{ s}^{-1}$) than for APO-UNUSUS rats [$480 \pm 58 \text{ bpm}\cdot 80 \text{ s}^{-1}$; $F(1, 15) = 9.19, p < 0.01$], due to the (twice as) long duration of the heart rate response to the electrical stimulus. This stimulus resulted in the significantly higher intra-arterial systolic pressures (SAP) in APO-SUS rats ($154 \pm 5 \text{ mmHg}$) than in APO-UNUSUS rats [$135 \pm 5 \text{ mmHg}$; $F(1, 15) = 7.06, p = 0.018$]. Otherwise pre-propofol data did not differ between the two lines.

Propofol induced biphasic changes in heart rates and in IA pressures: a decrease followed by an increase of heart rate, systolic IA pressure and diastolic IA pressure. Variables for which significant changes between the two lines were found are shown in Figure 4; Table 3 provides an overview. The magnitude of the reductions in the heart rate differed between the two lines at the doses [dose: $F(6, 105) = 29.96, p < 0.0001$; line and dose: $F(1, 6, 105) = 5.06, p = 0.0001$]. The dose related increase of the heart rates differed between the two lines [dose: $F(6, 105) = 5.83, p < 0.0001$; line: $F(1, 105) = 6.45, p = 0.013$]. The variations ($_{\text{0}}^{\text{HR}}$) of the heart rate were reduced in a dose-dependent fashion, and to lesser extent in the APO-SUS rats [two-way ANOVA: dose: $F(6, 105) = 31.26, p < 0.0001$; line: $F(1, 105) = 17.1, p = 0.0001$].

Similar differences were found for the % change of the variables. The reduction in intra-arterial diastolic (DAP) and systolic pressures (SAP) was related to the dose. APO-SUS rats maintained higher i.a. pressures [two-way ANOVA: SAP: dose: $F(1, 115) = 215.6, p < 0.0001$; line: $F(1, 115) = 23.77, p < 0.0001$; DAP: dose: $F(1, 115) = 67.8, p < 0.0001$; line: $F(1, 115) = 23.23, p < 0.0001$]. The %change of the SAP was dose dependent as well, $F(1, 115) = 392.52, p < 0.0001$, and the % reduction was less in APO-SUS rats, dose: $F(1, 115) = 7.58, p = 0.007$. The % reduction of the DAP was dose dependent, $F(1, 115) = 88.15, p < 0.0001$, and was not different between the two lines, $F(1, 115) = 2.18, p = 0.13$. The augmentations of the IA pressures that followed initial reductions related to the dose and differed for the lines [SAP: dose: $F(6, 105) = 21.49, p < 0.0001$, line: $F(1, 105) = 9.03, p < 0.0001$; DAP: dose: $F(6, 105) = 14.95, p < 0.0001$, line: $F(1, 105) = 34.78, p < 0.0001$]. Stimulus-related variations of IA pressures

TABLE 2

PARAMETER ESTIMATES AND SEM AFTER FITTING THE PHARMACODYNAMIC MODEL TO THE DATA OF THE REFLEX SUPPRESSIVE EFFECTS IN EACH INDIVIDUAL RAT AFTER CONSECUTIVE INJECTIONS OF PROPOFOL

Line	γ	ED_{50} ($\text{mg}\cdot\text{kg}^{-1}$)	τ_{onset} (min)	τ_{decay} (min)
SUS	2.22 ± 0.22	1.07 ± 0.14	0.60 ± 0.09	12.7 ± 0.48
UNUSUS	1.87 ± 0.201	1.07 ± 0.14	0.50 ± 0.06	10.4 ± 0.79

SUS = APO-SUS rats; UNUSUS = APO-UNUSUS rats.

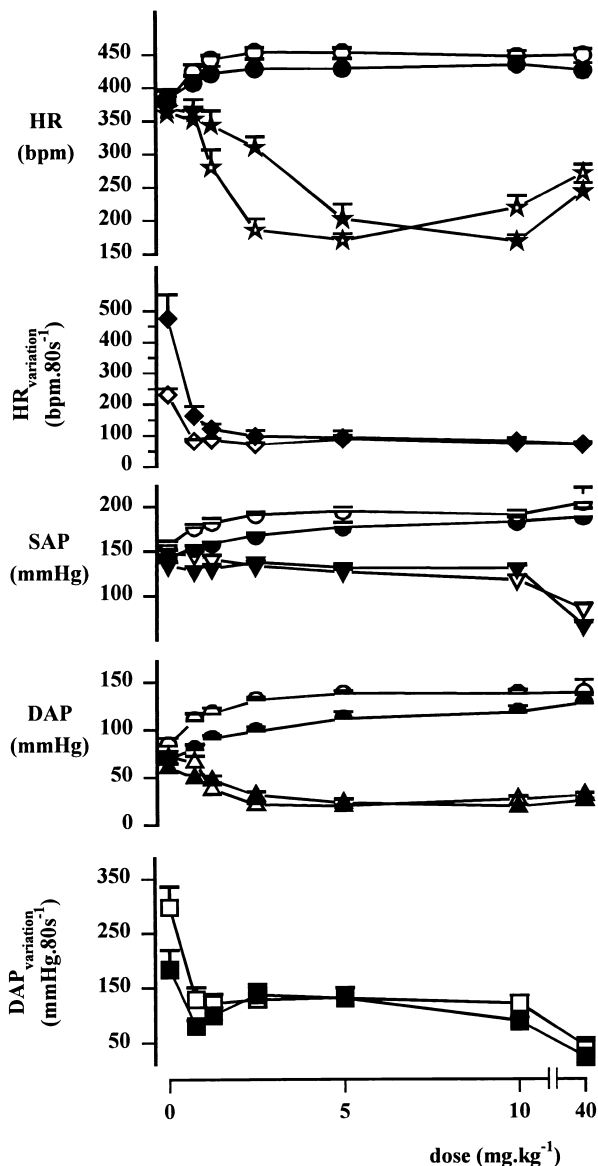


FIG. 4. Cardiovascular variables for APO-SUS (open symbols) and APO-UNUSUS rats (filled symbols) in relationship to the dose of propofol ($\text{mg}\cdot\text{kg}^{-1}$). Symbols represent the mean, bars the SEM (an overview of the changes is given in Table 3). HR = heart rate (bpm = beats per min); with reduction (\star) and increase (\circ) of the heart rate; and change of variation of the heart rate (\diamond). Intra-arterial pressures (mmHg), with increases (\circ) and decreases (∇) of the systolic IA pressure (SAP) and increases (\circ) and decreases (Δ) of the diastolic IA pressure (DAP); and change of the variation of the DAP (\square).

were dose-dependently reduced and to a greater extent in the APO-SUS rats [$_{0f}^{80}\text{SAP}$: dose: $F(6, 105) = 11.96, p < 0.0001$; line: $F(1, 105) = 7.56, p = 0.007$; $_{0f}^{80}\text{DAP}$: dose: $F(6, 105) = 16.78, p < 0.0001$; line: $F(1, 105) = 8.05, p = 0.005$; Figure 4]. The % change in relation to the dose did not differ between the two lines for the $_{0f}^{80}\text{SAP}$ [dose: $F(1, 115) = 27.11, p < 0.0001$; line: $F(1, 115) = 0.05, p = 0.8$]; whereas $_{0f}^{80}\text{DAP}$ differed for dose and line [dose: $F(1, 115) = 45.12, p < 0.0001$; line $F(1, 115) = 10.82, p = 0.0013$].

DISCUSSION

General

In the present study it was investigated to what extent the effects of the anesthetic propofol that is known to enhance indirectly the GABA transmission, vary in rats that show genetically determined differences in the cerebral GABA transmission, namely rats belonging to the APO-SUS and APO-UNUSUS Wistar rat lines. The main findings of this study are: (1) propofol induces narcosis and suppression of the withdrawal reflexes of the hindlimb of APO-SUS and APO-UNUSUS rats equally in a dose-dependent manner; (2) propofol elicited a number of behavioral signs that differ between the two lines of rats; and (3) propofol alters certain cardiovascular parameters which also differed between the two types of rats. These findings are discussed separately.

Narcosis and Withdrawal Reflexes

Narcosis was induced by propofol in APO-UNUSUS and APO-SUS rats, and this effect required similar doses. Analogous results were obtained in experiments assessing propofol-induced changes in withdrawal reflex. There were no significant differences between the two lines as far as dose dependent suppression of the reflex activity was concerned.

The absence of any line-specific difference in sensitivity to propofol's anesthetic effect may be explained in two ways: either the genetically determined differences in GABA transmission between APO-SUS and APO-UNUSUS rats do not significantly extend to those brain structures that mediate the anesthetic effect of propofol, and/or the anesthetic effect of propofol is only mediated via its ability to enhance the GABAergic transmission in the brain to a minor degree (1,9,21,32). In this context it is relevant to note that comparison of line-specific differences in the neostriatum, its first-order output station, namely the substantia nigra pars reticulata, and its second-order output station, namely the deeper layers of the colliculus superior, shows that these differences diminish increasingly (10). In other words, the line-specific differences become smaller as the brain region in question is further away from the telencephalon and closer to the brainstem and spinal cord. This is consistent with our data on the absence of a line-specific difference in propofol-induced reflex suppressive effects which are thought to be mainly mediated by structures in the brainstem and spinal cord.

Behavior

Propofol induced IVMCs in both lines, and the incidence of IVMCs was significantly higher in APO-SUS rats than in APO-UNUSUS rats. The origin of this sign is not known. Myoclonic jerks and muscle spasms can be induced by inhibiting the GABA transmission in the colliculus superior (Ellenbroek & Cools, unpublished data). Given the fact that there is an inverse relationship between the GABAergic transmission in the colliculus superior and the substantia nigra pars reticulata (5), this would imply that enhancing the GABA transmission in the substantia nigra pars reticulata might also induce myoclonic jerks. To our knowledge, this has so far not been studied. However, APO-SUS rats are characterized by a lower functional activity of GABA in the substantia nigra, pars reticulata (10) and, accordingly, are more sensitive to GABA than APO-UNUSUS rats. This together with the known facilitating effect of propofol upon GABA transmission suggests that the genetically determined differences in GABA

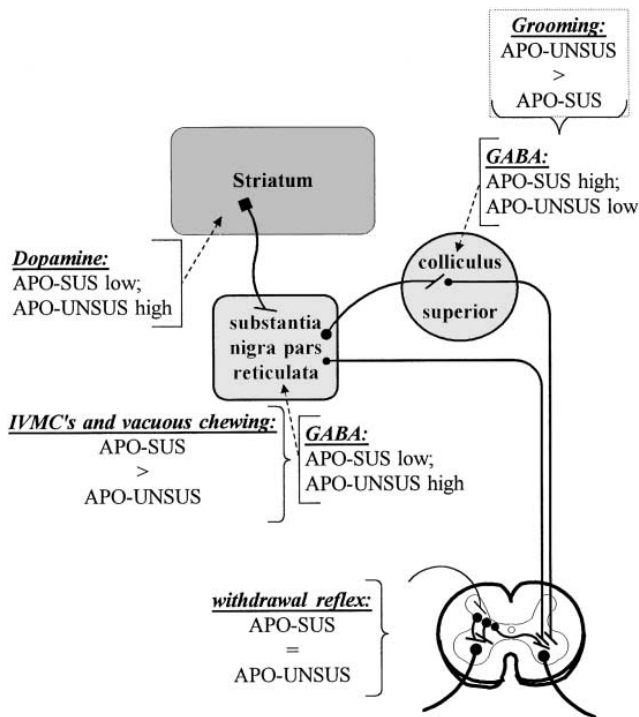


FIG. 5. Model of defined differences in GABA functionality within structures of the brain of APO-SUS and APO-UNUSUS rats and effects of propofol. The incidence of drug-induced behavior may be greater (>) or not different (=).

activity in the substantia nigra, pars reticulata (Figure 5) might have contributed to the line-specific difference in propofol-induced IVMCs.

Propofol induced oral movements, and the incidence of this sign was significantly higher in the APO-SUS rats than in the APO-UNUSUS rats: APO-SUS rats showed significantly more vacuous chewing with tongue protrusion than APO-UNUSUS rats did. The neural mechanisms underlying this effect are virtually unknown. In general, oral movements are subdivided into object oriented (like gnawing) and non-object ori-

ented (like vacuous chewing) mouth movements. Many studies have shown that the neuronal substrates for these two categories of oral movements are, at least partly, different. For instance, stimulation of GABA transmission in the substantia nigra, pars reticulata leads to object oriented gnawing (35), whereas inhibition of GABA transmission in the same structure leads to non-object oriented vacuous chewing movements (20). The oral movements observed in this study were non-object oriented. However propofol had caused general anesthesia as well and, thus, prevented the animals from displaying object oriented behavior. Taking this into account, it is possible that the genetically determined differences in GABA activity in the substantia nigra, pars reticulata might have contributed to the line-specific difference in propofol-induced oral movements (10).

Propofol induced grooming which mainly consisted of face washing. This study shows that APO-SUS rats showed significantly less propofol-induced grooming than APO-UNUSUS rats did. In awake unmedicated rats, this type of grooming is considered to be a displacement activity (36) and occurs when an animal is challenged by novelty (16). Grooming also occurs when the animal is falling asleep (2). These features may be brought about by the anesthetic: the novelty of literally falling asleep (narcosis). It is known that stimulation of GABA transmission in the colliculus superior enhances ACTH- and swimming-induced grooming (37; Ellenbroek & Cools unpublished data). APO-SUS rats are characterized by a higher functional activity of GABA in the colliculus superior (10) and are, accordingly, less sensitive to GABA than APO-UNUSUS rats at this level of the brain. This together with the fact that propofol enhances GABA transmission (1,9,21,32) suggests that genetically determined difference in GABA transmission at the level of the deeper layers of the colliculus superior might have contributed to the line-specific difference in propofol-induced grooming.

Cardiovascular Responses

APO-SUS rats had a lower baseline level of stimulus-induced heart rate variability as well as a greater variation in the diastolic IA pressure than APO-UNUSUS rats. The dose-related reduction of cardiovascular variables reduced the line-specific differences in level and stimulation-evoked variations of the cardiovascular variables, whereas the opposite occurred

TABLE 3
A COMPARISON OF THE CARDIOVASCULAR VARIABLES IN APO-SUS AND APO-UNUSUS RATS BEFORE INJECTIONS OF PROPOFOL AND THE DIFFERENCES IN THE DOSE-DEPENDENT REDUCTIONS OF THESE VARIABLES AFTER INJECTIONS OF PROPOFOL

Variable	Base Line	Propofol-Induced Reduction	
		Actual Change	% Change
HR level	SUS = UNSUS	SUS < UNSUS	SUS < UNSUS
variability	SUS < UNSUS	SUS < UNSUS	SUS < UNSUS
SAP level	SUS > UNSUS	SUS > UNSUS	SUS > UNSUS
variability	SUS = UNSUS	SUS > UNSUS	SUS = UNSUS
DAP level	SUS = UNSUS	SUS > UNSUS	SUS = UNSUS
variability	SUS > UNSUS	SUS > UNSUS	SUS > UNSUS

SUS = APO-SUS rats; UNSUS = APO-UNUSUS rats; = not different; > larger than ($p < 0.05$); < less than ($p < 0.05$).

The actual changes in relationship to the dose are shown in Fig. 4.

during the vanishing of the effect. In fact, propofol-treated APO-SUS rats displayed a greater cardiovascular responsiveness with higher rebound heart rates and intra-arterial pressures than APO-UNSUS rats. Apart from the fact that APO-SUS are known to be far more sensitive to stressor-induced behavioral responses than APO-UNSUS rats (10), APO-SUS rats also have lower plasma levels of free corticosteroids during rest conditions and have a greater release of ACTH, corticosteroids and noradrenaline and a lower release of adrenaline in response to mild stressors compared to APO-UNSUS rats (33; Rots et al., personal communications). The noted line-specific baseline differences in cardiovascular parameters as well as the line-specific differences in response to propofol fully fit in with these line-specific differences in sensitivity to stressors. Finally, genetically determined differences in the GABA transmission at supraspinal level have been found to be associated with differences in cardiovascular responses (17). To what extent the known line-specific difference in the GABA-ergic make-up of the forebrain has also contributed to the noted line-specific differences in cardiovascular responses remains to be investigated.

CONCLUSION

The present study shows that genetically determined differences in cerebral GABA transmission, especially in the striato-nigro-collicular pathway, do not give rise to differences in the effect of the anesthetic propofol upon narcosis and suppression of the hindlimb withdrawal reflex. Since the rats under study are marked by other genetically determined differences

in the make-up of the brain (10,11,12) which may theoretically mask the role of the GABA-ergic striato-nigro-collicular pathway, we cannot exclude a role for this pathway in these effects. This study suggests that genetically determined differences in the GABA-ergic striato-nigro-collicular pathway give rise to differences in the effect of propofol upon certain cardiovascular and behavioral parameters, although it cannot yet be excluded that additional, genetically determined differences may be involved as well.

The genetically determined difference in the GABA transmission between APO-SUS and APO-UNSUS rats is only one of many features that are characteristic for these animals (11,12). They are no mutants, but occur normally in every unselected outbred strain of Wistar rats. Each type has its own individual-specific hardware and software to cope with the challenges from the internal and external environment, and has its own vulnerability to therapeutic and unwanted side-effects of centrally acting drugs (11). The present study extends these data by showing that the greater vulnerability of APO-SUS rats for propofol-induced IVMCs and vacuous chewing co-exist with particular baseline features of the cardiovascular system. In other words, these cardiovascular features which co-exist with particular behavioral features (12), may represent biological features that can be used to predict to what extent rats will display these side-effects in response to propofol. Extrapolating the present findings to man and other anesthetics results in the notion that both cardiovascular and behavioral/psychological characteristics may be useful for predicting to what extent anesthetics can elicit unwanted side-effects in the individual patient.

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