The following full text is a publisher’s version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/24815

Please be advised that this information was generated on 2020-01-11 and may be subject to change.
Responses to Propofol in Relation to GABA Functionality of Discrete Parts of the Brain of Rats

RIS DIRKSEN,* BART ELLENBROEK,† JAN VAN EGMOND* AND ALEXANDER R. COOLS†

*Departments of Anesthesiology and †Psychoneuropsychopharmacology, University of Nijmegen, the Netherlands

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.
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 × 24 × 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 × 80 × 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 transcuscutaneous bipolar stimulation (Grass stimulator S11, with stimulus isolation unit SIUSA, 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 Hz (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-61LT, 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 mg.kg−1; IV; n = 3/10 mg.kg−1; IV; n = 2/20 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 mg.kg\(^{-1}\) of propofol, thereby including subanesthetic and anesthetic doses, and the midst of two stimuli (40 s after a 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 \(\Delta F_{\text{peak}}\) (\(\Delta F/\Delta t\)) with \(\Delta t\) = interval (min) between stimulus and maximum withdrawal], and for relaxation [the \(\Delta F_{\text{relax}}\) (\(\Delta F/\Delta t\)) with \(\Delta t\) = interval (min) between max. withdrawal and return to resting value]. The time course of reflex suppressive effect was assessed in terms of time constants (\(\tau_{\text{on}}\) min) of onset (\(\tau_{\text{on}}\)) and decay (\(\tau_{\text{dec}}\)) 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 in data analysis. In Figure 1b, the effect of 10 mg.kg\(^{-1}\) propofol in the same rat is shown to illustrate how the effects of propofol on these indices were analyzed.

**STANDARD 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 \(\Delta F_{\text{peak}}\) (\(\Delta F/\Delta t\)), with \(\Delta t\) = interval (min) between stimulus and maximum withdrawal, and for relaxation [the \(\Delta F_{\text{relax}}\) (\(\Delta F/\Delta t\)) with \(\Delta t\) = interval (min) between max. withdrawal and return to resting value]. The time course of reflex suppressive effect was assessed in terms of time constants (\(\tau_{\text{on}}\) min) of onset (\(\tau_{\text{on}}\)) and decay (\(\tau_{\text{dec}}\)) 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 in data analysis. In Figure 1b, the effect of 10 mg.kg\(^{-1}\) propofol in the same rat is shown to illustrate how the effects of propofol on these indices were analyzed.

**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 Scheffes 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 equation to the data yielded three parameter estimates: \(E_{\text{max}} = \) maximum effect, \(\gamma = \) 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

<table>
<thead>
<tr>
<th>Dose</th>
<th>Injection</th>
<th>Incidence</th>
<th>Duration (mean ± SE)</th>
<th>Incidence</th>
<th>Duration (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>first</td>
<td>0</td>
<td>0</td>
<td>22</td>
<td>0.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>consecutive</td>
<td>88</td>
<td>5.7 ± 0.7</td>
<td>100</td>
<td>7.3 ± 0.4</td>
</tr>
<tr>
<td>10</td>
<td>first</td>
<td>77</td>
<td>7.8 ± 1.5</td>
<td>100</td>
<td>9.7 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>consecutive</td>
<td>100</td>
<td>13.3 ± 0.3</td>
<td>100</td>
<td>11.9 ± 0.6</td>
</tr>
<tr>
<td>20</td>
<td>first</td>
<td>100</td>
<td>16.2 ± 0.9</td>
<td>100</td>
<td>17.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>consecutive</td>
<td>100</td>
<td>18.0 ± 0.9</td>
<td>100</td>
<td>22.3 ± 1.3</td>
</tr>
</tbody>
</table>

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 [χ² (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 = 0.014 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 [χ² (28.29), p < 0.001; post hoc: 4 mg.kg⁻¹ different from 10 and 20 mg.kg⁻¹; p < 0.001 and < 0.01, 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 [χ² (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.
Propofol-Induced Changes in Withdrawal Reflex of APO-SUS and APO-UNSUS 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) = 3.8, p = 0.05; 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 parameter estimates and standard errors of the estimates for γ and ED90 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); Emax maximized at 100%.

Although there was a slight but significant difference of the tdecay of the pharmacological response between APO-SUS rats and APO-UNSUS 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-dependent fashion, and these effects, or the change in terms of percentage did not differ between APO-SUS and APO-UNSUS rats (data not shown). After the return of the force of withdrawal to pre-propofol levels, peak (Δforce/Δt) was enhanced in a dose related fashion, F(1,115) = 5.46, p = 0.001, 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 (ΔHR) was significantly less for APO-SUS rats (230 ± 55 bpm.80 s⁻¹) than for APO-UNSUS rats [480 ± 58 bpm.80 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 ± 5 mmHg) than in APO-UNSUS rats [135 ± 5 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 (γ) 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 = 0.001].

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 this effect was not different between the two lines, 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

<table>
<thead>
<tr>
<th>Line</th>
<th>γ</th>
<th>ED90 (mg.kg⁻¹)</th>
<th>tdecay (min)</th>
<th>τmax (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUS</td>
<td>2.22 ± 0.22</td>
<td>1.07 ± 0.14</td>
<td>0.60 ± 0.09</td>
<td>12.7 ± 0.48</td>
</tr>
<tr>
<td>UNSUS</td>
<td>1.87 ± 0.201</td>
<td>1.07 ± 0.14</td>
<td>0.50 ± 0.06</td>
<td>10.4 ± 0.79</td>
</tr>
</tbody>
</table>

SUS = APO-SUS rats; UNSUS = APO-UNSUS rats.
were dose-dependently reduced and to a greater extent in the APO-SUS rats [\( F_{1,115}^{\text{SAP}} \) dose: \( F(6, 105) = 11.96, p < 0.0001 \); line: \( F(1, 105) = 7.56, p = 0.007 \); \( F_{1,115}^{\text{DAP}} \) 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 \( F_{1,115}^{\text{SAP}} \) [dose: \( F(1, 115) = 27.11, p < 0.0001 \); line: \( F(1, 115) = 0.05, p = 0.8 \)]; whereas \( F_{1,115}^{\text{DAP}} \) differed for dose and line [dose: \( F(1,115) = 45.12, p < 0.0001 \); line \( F(1, 115) = 10.82, p = 0.0013 \].

**DISCUSSION**

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-UNSUS 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-UNSUS 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-UNSUS 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-UNSUS 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-UNSUS 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 (5), this might 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-UNSUS rats. This together with the known facilitating effect of propofol upon GABA transmission suggests that the genetically determined differences in GABA...
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-UNSUS rats. APO-SUS rats showed significantly more vacuous chewing with tongue protrusion than APO-UNSUS 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 oriented (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-UNSUS 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-UNSUS 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-UNSUS 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**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Base Line</th>
<th>Propofol-Induced Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR level variability</td>
<td>SUS = UNSUS</td>
<td>SUS &lt; UNSUS, SUS &lt; UNSUS</td>
</tr>
<tr>
<td>SAP level variability</td>
<td>SUS &gt; UNSUS</td>
<td>SUS &gt; UNSUS, SUS &gt; UNSUS</td>
</tr>
<tr>
<td>DAP level variability</td>
<td>SUS = UNSUS</td>
<td>SUS &gt; UNSUS, SUS &gt; UNSUS</td>
</tr>
</tbody>
</table>

SUS = APO-SUS rats; UNSUS = APO-UNSUS 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 adrenaline 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-colicular 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-colicular 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-colicular 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 not 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.

REFERENCES


