Evaluation and comparison of the pharmacokinetic and pharmacodynamic properties of allopregnanolone and pregnanolone at induction of anaesthesia in the male rat

D. Zhu¹, M. D. Wang¹, T. Bäckström¹ and G. Wahlström²*

¹Department of Clinical Science, Section of Obstetrics and Gynaecology, University of Umeå, S-901 87 Umeå, Sweden. ²Department of Pharmacology and Clinical Neuroscience, University of Umeå, S-901 87 Umeå, Sweden

*Corresponding author

We have evaluated and compared the pharmacokinetic and pharmacodynamic properties of allopregnanolone and pregnanolone at induction of anaesthesia in male rats. A threshold method was used, and the first burst suppression period of 1 s or more in the EEG was selected as the end-point after fairly slow infusions. An optimal dose of 4.0 mg kg⁻¹ min⁻¹ was noted for both steroids. Brain concentrations were low at low infusion rates, indicating that acute tolerance was not occurring. Significant positive correlations were noted between dose rate and serum concentrations of allopregnanolone (r = 0.94, P < 0.001) and pregnanolone (r = 0.88, P < 0.001). Such correlations were also seen in striatum, cerebellum, cortex and muscle for both steroids (P < 0.01). Despite changing infusion rates, the concentrations of both steroids in brainstem, hippocampus and fat remained stable. Because no correlation between infusion rate and steroid concentration was noted in the brainstem and hippocampus, these two brain areas may be regarded as primary sites of action for allopregnanolone and pregnanolone. Pregnanolone concentrations in the brainstem and hippocampus were significantly higher than those of allopregnanolone, suggesting that allopregnanolone was more potent than pregnanolone in inducing anaesthesia.

Br J Anaesth 2001; 86: 403–12

Keywords: pharmacokinetics, allopregnanolone; pharmacokinetics, pregnanolone; pharmacodynamics, allopregnanolone; pharmacodynamics, pregnanolone; anaesthesia; monitoring, electroencephalography; brain, brainstem; brain, hippocampus

Accepted for publication: September 25, 2000

The major ring-A-reduced metabolites of progesterone, allopregnanolone (3α-hydroxy-5α-pregnane-20-one) and pregnanolone (3α-hydroxy-5β-pregnane-20-one) are barbiturate-like anaesthetics whose site of action is the GABA<sub>A</sub> receptor.¹ ² They are the most potent naturally occurring anaesthetics tested so far in both animals and humans.³ ⁴ When tested with an i.v. EEG threshold method in male rats,³ the anaesthetic potencies of allopregnanolone and pregnanolone are greater than those of other i.v. anaesthetics that are or have been used in the clinic (Table 2).

In an earlier study,⁵ we found that the overall brain concentrations of allopregnanolone at induction of anaesthesia were significantly increased in spite of the observation that the threshold dose did not differ when the infusion rate was increased from 2.0 to 3.0 mg kg⁻¹ min⁻¹. Similarly, a significant increase in brain concentration (decrease in brain sensitivity) in relation to increased infusion rate has also been observed earlier with thiobutabarbital and butabarbital.⁶ Earlier results with hexobarbital indicated that a criterion of critical brain areas with respect to induction of anaesthesia should be no change in active drug concentration with changing infusion rate.⁶ However, an increase in hexobarbital concentration occurred in all brain areas at a slow infusion rate, indicating a decrease in brain sensitivity. This was a sign of acute tolerance to hexobarbital, as higher brain concentrations were needed to induce anaesthesia when a longer infusion time was used.⁷

Acute tolerance is the induction of functional tolerance, i.e. altered sensitivity to a drug, within the duration of a single continuous exposure to a drug.⁸ Since it was first investigated,⁹ the interest in acute tolerance has focused on ethanol.¹⁰–¹³ However, it has also been described for many classes of general anaesthetics, including barbiturates,¹⁴ volatile anaesthetics¹⁵ and benzodiazepines.¹⁶ Several neuro-
transmitter systems, such as dopamine, noradrenaline, 5-hydroxytryptamine, acetylcholine, GABA and NMDA, have been implicated directly or indirectly in the development of acute tolerance. This makes it uncertain whether acute tolerance to depressant drugs in the central nervous system (CNS) is an entity with a single common mechanism. Furthermore, the development of acute tolerance varied greatly among different brain structures depending on the test system used and the age of the experimental animals.

A simple method of investigating the development of acute tolerance to i.v. anaesthetics is to increase the duration of induction by changing the rate of infusion of the drug. Acute tolerance is revealed by comparing drug concentrations in the brain at the occurrence of a defined criterion of anaesthesia. In the present study, we used brain concentrations of allopregnanolone and pregnanolone at the time of an EEG end-point induced at different rates as a measure of CNS sensitivity. This design also made it possible to compare the pharmacodynamic properties of allopregnanolone and pregnanolone at the induction of deep anaesthesia in male rats, and to identify the critical brain areas involved in the induction of anaesthesia with allopregnanolone and pregnanolone.

Materials and methods

Animals

The study was approved by the Regional Ethics Committee for Animal Experiments in Umeå (Umeå djurförsöksnämnd). Male adult Sprague-Dawley rats (n = 47) (MOL: SPDR HAN, Möllegaard, Li, Skensved, Denmark) were used in all experiments. The rats were housed at three per cage in a room with constant temperature (24°C). They had access to water and standard rat food ad libitum.

Drugs

Allopregnanolone (CoCensys, Irvine, CA, USA) and pregnanolone (Steraloids, Wilton, NH, USA) were dissolved in 20% 2-hydroxypropyl-β-cyclodextrin (β-cyclodextrin; Sigma, St Louis, MO, USA) at a concentration of 4.0 mg ml⁻¹. The preparations were placed in a Bransonic 2210 ultrasonic bath for approximately 15 h and agitated occasionally. All steroids were dissolved in β-cyclodextrin by visual inspection.

EEG threshold method

The anaesthetic effects of allopregnanolone and pregnanolone in the rats were determined with an i.v. EEG threshold method. Allopregnanolone or pregnanolone was infused i.v. into one tail vein at a constant infusion rate, and the EEG was recorded continuously from subcutaneous stainless steel electrodes. The infusion was stopped immediately when the first burst suppression period of 1 s or more was noted in the EEG (the ‘silent second’, SS). As illustrated in earlier papers, the SS is easily detected against the background of an EEG consisting mainly of high-amplitude potentials. The appearance of the SS occurs at a much deeper level of anaesthesia than the loss of the righting reflex. The time to reach the SS was recorded and the amount of steroid needed to induce the effect was calculated. This dose was considered to be the threshold dose. Data for a dose rate curve were generated by repeated determinations with different infusion rates. Allopregnanolone was infused at 1.0 (n = 1), 2.0 (n = 5), 4.0 (n = 5) and 8.0 (n = 6) mg kg⁻¹ min⁻¹, and pregnanolone at 0.5 (n = 3), 1.0 (n = 6), 2.0 (n = 6), 4.0 (n = 6) and 8.0 (n = 9) mg kg⁻¹ min⁻¹. The order of testing and dose rates were allocated at random. However, because of failure to induce anaesthesia with the lower rates (see Results, Dose rate curves for allopregnanolone and pregnanolone at induction of SS), not all rats were used. A few of the unused rats were reallocated to the highest dose rate. The infusion dose rate that gave the lowest threshold dose when drug administration was terminated at SS was defined as the optimal dose rate.

Tissue sample preparation

Rats were killed by decapitation after the first SS was initiated. Trunk blood was collected and the brain was dissected immediately into the cerebellum, cortex, hippocampus, brainstem (midbrain, medulla oblongata) and striatum, largely according to Glowinski and Iversen. The blood vessels on the surface of the brain hemispheres were carefully removed. A macroscopic autopsy was always carried out. Abdominal fat tissue from the retroperitoneal areas and part of the psoas muscle were removed from each rat. After weighing, the tissue was frozen at −70°C until analysis. All the samples were extracted later with 99.5% ethanol for 7 days at 4°C. Recovery of steroids in this procedure has been shown to be 100%.

Celite chromatography and steroid assay

Allopregnanolone and pregnanolone in tissue and serum extracts were separated by Celite chromatography, as described by Bäckström et al. and verified by Corpechot et al. The recovery rate of allopregnanolone and pregnanolone was determined in every run by using [1H]allopregnanolone and [1H]pregnanolone tracers. Recovery of allopregnanolone and pregnanolone was 85%. The concentrations of allopregnanolone and pregnanolone in brain tissue extracts were measured by radiimmunoassay. Radioactive steroid tracers ([9,11,12-3H(N)]-5α-pregnan-3α-ol-20-one and [9,11,12-3H(N)]-5β-pregnan-3α-ol-20-one) were purchased from NEN Life Science Products, Boston, MA, USA. Allopregnanolone and pregnanolone antisera were raised against 3α-hydroxy-
20-oxo-5α-pregnan-11α-yl carboxymethyl ether or 3α-hydroxy-20-oxo-5β-pregnan-11α-yl carboxymethyl ether coupled to bovine serum albumin. Antiserum was kindly provided by Dr Robert Purdy (San Diego, CA, USA). Cross-reactivity has been tested earlier, and both antisera are highly specific. The sensitivity of both assays was 25 pg, with an intra-assay coefficient of variation of 7% and an inter-assay coefficient of variation of 8%. The intra-assay coefficient of variation was calculated from duplicate values in the assay. The mean inter-assay control for pregnanolone was 20.6 nmol g⁻¹ (range 18.2–26.4 nmol g⁻¹) and for allopregnanolone it was 13.1 nmol g⁻¹ (range 11.2–22.5 nmol g⁻¹).

**Statistical analysis**

Differences in the threshold doses among dose rate groups and between allopregnanolone and pregnanolone concentrations in serum and tissue samples were assessed with the Mann–Whitney U-test. Parametric correlations (r) and regression coefficients (b) were used to study the relationship between infusion dose rate and steroid concentrations and the relationship between threshold doses and steroid concentrations respectively. All statistical calculations were performed using Origin version 4.1 software (Microcal Software, Northampton, MA, USA). Values of \( P < 0.05 \) in a two-tailed test were taken to represent a significant difference. All results are presented as mean (SEM). The symbol \( n \) denotes the number of animals in each test.

**Results**

*Dose rate curves for allopregnanolone and pregnanolone at induction of SS*

The dose rate curve (Fig. 1A) shows the dose for SS plotted against the infusion rate for allopregnanolone and pregnanolone. A V-shaped curve was obtained for both steroids. For both curves, an optimal infusion rate of 4.0 mg kg⁻¹ min⁻¹ was found which gave the lowest dose of allopregnanolone or pregnanolone needed to obtain an SS (i.e. the threshold dose). The corresponding optimal threshold doses were 14.05 (1.32) mg kg⁻¹ for allopregnanolone and 10.46 (0.37) mg kg⁻¹ for pregnanolone. The threshold doses of pregnanolone were significantly lower than those of allopregnanolone at the infusion rates of 2.0, 4.0 and 8.0 mg kg⁻¹ min⁻¹ (\( P < 0.001 \), Mann–Whitney U-test).

In one rat tested with allopregnanolone at 1.0 mg kg⁻¹ min⁻¹, no SS was obtained after 29.5 min of infusion, when a dose of 29.5 mg kg⁻¹ had been injected. Among the three rats tested with pregnanolone at 0.5 mg kg⁻¹ min⁻¹, SS was obtained in only one rat; for the other two rats, the infusion was stopped after 50 min, when a dose of 25 mg kg⁻¹ pregnanolone had been injected, with no sign of an SS in the EEG. Results from one rat that received pregnanolone 8.0 mg kg⁻¹ min⁻¹ were excluded because of changes in the lungs.
Serum concentrations of allopregnanolone and pregnanolone plotted against dose rate are shown in Fig. 1B. A significant linear increase in serum concentrations with increasing infusion rate was noted for allopregnanolone \([b = 7.55, \ r = 0.94, \ df (\text{degrees of freedom}) = 14, \ P < 0.001]\) and pregnanolone \([b = 7.33, \ r = 0.88, \ df = 24, \ P < 0.001]\). Although the threshold doses for allopregnanolone at induction of SS were significantly higher than for pregnanolone (Fig. 1A), the average serum concentrations of the two steroids were not significantly different at any of the infusion rates tested.

The relationships between dose rate and tissue concentration at SS are shown in Fig. 2A and B for allopregnanolone and pregnanolone respectively. For allopregnanolone, cortex \([b = 2.3, \ r = 0.77, \ df = 14, \ P = 0.001]\), striatum \([b = 1.44, \ r = 0.60, \ df = 14, \ P = 0.014]\), cerebellum \([b = 2.16, \ r = 0.75, \ df = 14, \ P = 0.001]\) and muscle \([b = 1.64, \ r = 0.71, \ df = 14, \ P = 0.002]\) showed significantly increased steroid concentration at SS with increasing infusion rate (Fig. 2A). However, the allopregnanolone concentrations in brainstem, hippocampus and fat did not change significantly with infusion rate.

Tissue concentrations of pregnanolone at SS showed essentially the same pattern as those of allopregnanolone when plotted against dose rate (Fig. 2B). A significant increase in pregnanolone concentration with increasing infusion rate was noted in cortex \([b = 1.53, \ r = 0.68, \ df = 24, \ P < 0.001]\), striatum \([b = 1.63, \ r = 0.63, \ df = 24, \ P = 0.001]\), cerebellum \([b = 2.22, \ r = 0.65, \ df = 24, \ P < 0.001]\) and muscle \([b = 1.53, \ r = 0.69, \ df = 24, \ P < 0.001]\). No significant change in pregnanolone concentration in relation to increasing infusion rate was noted in brainstem, hippocampus and fat. These results indicated that allopregnanolone and pregnanolone concentrations increased in the cortex, striatum and cerebellum with increasing infusion rate. However, no changes in steroid concentrations in brainstem and hippocampus occurred at SS in relation to dose rate. As no changes in active drug concentrations were expected at the primary sites of action in the brain, the brainstem and hippocampus can be considered to be the primary sites of action with respect to allopregnanolone and pregnanolone in male rats.

Relationship between threshold dose and tissue concentration

The relationships between threshold dose and steroid concentration in different tissues were assessed by linear regression. In the analysis of allopregnanolone, no significant relationship was found for serum, muscle, cortex, striatum, cerebellum and hippocampus. A significant relationship was found for brainstem \([b = 0.90, \ r = 0.57, \ df = 14, \ P < 0.05]\) (Fig. 3A) and fat \([b = 1.31, \ r = 0.69, \ df = 14, \ P < 0.001]\).
The analysis for pregnanolone gave similar results. No significant relationship was noted between threshold dose and steroid concentration in serum, muscle, cortex, striatum, cerebellum and hippocampus, whereas a significant correlation was found for brainstem ($b = 1.29$, $r = 0.52$, $df = 24$, $P = 0.001$) (Fig. 3c) and fat ($b = 1.21$, $r = 0.45$, $df = 24$, $P < 0.05$) (Fig. 3d). These findings indicate that the brainstem has a different role in the generation of anaesthesia in comparison with the hippocampus. Fat turns out to be a special tissue that handles allopregnanolone and pregnanolone actively, in a similar way to the brainstem.

**Comparison of steroid concentrations in brainstem and hippocampus**

As our results revealed that steroid concentrations in the brainstem and hippocampus did not change significantly with infusion rate, we used these two brain areas to determine the anaesthetic potencies of allopregnanolone and pregnanolone by comparing the steroid concentrations in these two brain areas (Table 1). Comparison of the active drug concentrations in the brain areas involved critically in the induction of SS is more informative than comparison of the threshold doses at SS, because the former eliminates the pharmacokinetic factors involved in the infusion of the drug.

---

**Fig 3** Relationship between threshold doses of allopregnanolone and concentrations in brainstem (A) and fat (B) obtained at the silent second. Relationship between threshold doses of pregnanolone and concentrations in brainstem (C) and fat (D) obtained at the silent second.
Table 1 Tissue concentrations of allopregnanolone and pregnanolone. Data are mean (SEM). NS = not significant.

<table>
<thead>
<tr>
<th>Infusion rate (mg kg⁻¹ min⁻¹)</th>
<th>Allopregnanolone (nmol g⁻¹)</th>
<th>Pregnanolone (nmol g⁻¹)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brainstem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>22.78 (2.35)</td>
<td>29.34 (2.04)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4.0</td>
<td>20.71 (1.96)</td>
<td>29.88 (1.94)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8.0</td>
<td>23.18 (3.13)</td>
<td>33.06 (1.78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average</td>
<td>22.28 (1.44)</td>
<td>30.71 (0.92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hippocampus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>20.47 (1.50)</td>
<td>23.40 (1.81)</td>
<td>NS</td>
</tr>
<tr>
<td>4.0</td>
<td>19.71 (1.15)</td>
<td>26.95 (1.40)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>8.0</td>
<td>19.02 (1.25)</td>
<td>25.26 (1.67)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Average</td>
<td>19.69 (0.72)</td>
<td>24.76 (0.83)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>23.96 (2.63)</td>
<td>15.23 (2.29)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4.0</td>
<td>20.33 (2.36)</td>
<td>13.36 (2.54)</td>
<td>NS</td>
</tr>
<tr>
<td>8.0</td>
<td>18.59 (2.56)</td>
<td>14.98 (2.68)</td>
<td>NS</td>
</tr>
<tr>
<td>Average</td>
<td>23.03 (2.59)</td>
<td>14.57 (1.42)</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

We found that pregnanolone concentration in the brainstem was significantly higher than the allopregnanolone concentration at the infusion rates of 2.0, 4.0 and 8.0 mg kg⁻¹ min⁻¹ (P<0.001, Mann–Whitney U-test). The average pregnanolone concentration when all infusion rates were pooled was also significantly higher than that of allopregnanolone (P<0.001). In the hippocampus, the pregnanolone concentration at the infusion rate of 2.0 mg kg⁻¹ min⁻¹ was not significantly different from the corresponding allopregnanolone concentration, while the pregnanolone concentrations at the infusion rates of 4.0 and 8.0 mg kg⁻¹ min⁻¹ were significantly higher than the corresponding allopregnanolone concentrations (P<0.05 for both infusion rates). When all infusion rates were pooled, the average pregnanolone concentration in hippocampus was significantly higher than that of allopregnanolone (P<0.001). A similar difference was seen in fat when all infusion rates were pooled (Table 1).

Relationship between serum and tissue concentrations

Data on serum concentrations are given in Fig. 1B; data on tissue concentrations of allopregnanolone are given in Fig. 2A and those of pregnanolone are given in Fig. 2B. Dose rate was used as the independent variable in both sets of data, and for both steroids there was a strong positive correlation with serum concentration (Fig. 1B). In some but not all tissues (Fig. 2A and B), positive correlations were also recorded. In these tissues, positive correlations with serum concentrations were expected. We found that the steroid concentrations in serum and muscle were significantly correlated for both allopregnanolone and pregnanolone (allopregnanolone, b = 0.18, r = 0.68, df = 14, P = 0.004; pregnanolone, b = 0.16, r = 0.59, df = 24, P = 0.001), while the correlation between serum and fat was not significant for any of the steroids. Serum concentrations of allopregnanolone were also correlated significantly with concentrations in striatum (b = 0.22, r = 0.75, df = 14, P = 0.001), cerebellum (b = 0.26, r = 0.72, df = 14, P = 0.002) and cortex (b = 0.27, r = 0.73, df = 14, P = 0.001). Serum concentrations of pregnanolone were significantly correlated with concentrations in striatum (b = 0.18, r = 0.57, df = 24, P = 0.002), cerebellum (b = 0.28, r = 0.69, df = 24, P<0.001) and cortex (b = 0.18, r = 0.68, df = 24, P<0.001).

Discussion

Sixty years ago, Selye28 first reported the sedative–anaesthetic activities of progesterone and deoxycorticosterone and identified their ring-A-reduced metabolites as extremely potent sedative–hypnotic agents. Selye’s initial observations on the anaesthetic effects of steroids, particularly the 3α-hydroxy ring-A-reduced metabolites of progesterone, prompted the development of a class of steroid anaesthetics that culminated in the introduction of several of them into clinical practice.2 29 The rapid onset of their behavioural effects precludes a non-genomic mechanism of action for such steroids. However, it was not until Harrison and Simmonds30 demonstrated in 1984 that a steroid anaesthetic, alphaxalone (3α-hydroxy-5α-pregn-11,20-dione), selectively enhanced the interaction of GABA with the GABA_A receptor, that a protein-founded mechanism to explain the behavioural effects of these compounds emerged. The natural 3α-hydroxy ring-A metabolites of progesterone, allopregnanolone and pregnanolone, have been shown to be the most potent GABA_A receptor ligands, with affinities comparable to those of benzodiazepines and at least a thousand times greater than that of the barbiturate pentobarbital.2 31 As for hexobarbital and thiopental, an increase in the sensitivity of the brain to allopregnanolone and pregnanolone with age has been observed.21 32 Because of these findings, the male rats in the present study were used at the age of 65–70 days, when their anaesthetic sensitivity to allopregnanolone and pregnanolone would have stabilized.32

The dose rate curves obtained with allopregnanolone and pregnanolone both have the same general appearance (Fig. 1A). The appearance of the curves is also similar to that of curves obtained with different barbiturates21 34 and some other i.v. anaesthetics.35 36 A number of threshold doses at optimal rates and the slopes of the curves at infusion rates higher than the optimal rate are included in Table 2. The table shows that there is large variation in threshold dose at the corresponding optimal rates. Both steroids are potent, with low threshold doses, but the solvent has an effect. Among other drugs that have been tested, only propofol and methohexitol have threshold doses at the optimal infusion rates that are within the same range as those of the steroids, but they seem to be slightly less potent.
There is also a distinct difference in threshold dose between the isomers of both hexobarbital and narcobarbital, the dose of the racemate being intermediate. Furthermore, there is considerable variation in the slope of rates higher than optimal (Table 2). If only simple diffusion at the site of action were involved in this slope, i.v. anaesthetics, which are highly lipid-soluble substances, would have a slope that corresponds to the circulation time. This seems to be the case for hexobarbital, methohexital, propofol and the two steroids, when tested in young rats. All other substances had slopes above 0.5 min, and among these it has been shown that the penetrations of thiopental into the brain could be facilitated by probenecid37 and penicillins.38 This indicates the presence of a transport mechanism, but for thiobutabarbital6 a change in sensitivity may also be involved. The rate curve clearly shows that, when the potencies of i.v. anaesthetics (and probably also of other substances) are compared in vivo, the dose obtained at the optimal infusion rate should be chosen. However, it is obvious that pharmacokinetic properties still have to be considered. The different results with different solvents in the case of steroids are only one example of remaining kinetic influences.

We found that less pregnanolone than allopregnanolone was needed to induce the SS in all comparable dose rate groups (Fig. 1A). However, serum concentrations of the two substances were almost identical (Fig. 1B). From a pharmacokinetic point of view, this indicates that there had been a rapid loss of allopregnanolone from the blood. Such a loss could be accomplished by rapid accumulation in some other tissues or by rapid metabolism of allopregnanolone. Redistribution to muscle, as proposed by Goldstein and Aronow,39 is unlikely because no difference between pregnanolone and allopregnanolone concentrations could be established. As seen in three barbiturates,6 fat is another alternative site of redistribution, especially after long induction times. In the present experiments there was a difference between the levels of accumulation of allopregnanolone and pregnanolone in fat (Table 1), but the difference was not large enough to explain the difference in threshold doses at SS (Fig. 2A). Furthermore, there was only a slight accumulation of allopregnanolone in fat at lower infusion rates (Fig. 2A). Such a result was unexpected and could indicate rapid loss of steroid from fat, but as not all tissues were analysed no definite conclusion can be drawn to explain the loss of allopregnanolone during infusion.

An earlier study7 has shown that acute tolerance to hexobarbital can be induced by stable anaesthesia lasting more than 10 min. This was in the same range as the infusion time for pregnanolone at infusion rates of 0.5 and 1.0 mg kg⁻¹ min⁻¹ (>10 min). For allopregnanolone the infusion time used to induce the SS at an infusion rate of 2.0 mg kg⁻¹ min⁻¹ was about 9 min. For the rat infused with allopregnanolone at 1.0 mg kg⁻¹ min⁻¹, an SS had not been obtained when the infusion time reached 29.5 min. This

Table 2  Doses at optimal infusion rates and slopes of the infusion rate curve at higher rates for various substances. All infusion rate curves were similar to those shown in Fig. 1A. The barbiturates were used as sodium salts. C=β-cyclodextrin; A=albumin; I=Intralipid; W=water; Rac=racemate; ND=not determined

<table>
<thead>
<tr>
<th>Substance</th>
<th>Dose at optimal rate [mg kg⁻¹ (mmol g⁻¹)]</th>
<th>Optimal infusion rate [mg kg⁻¹ min⁻¹]</th>
<th>Solution</th>
<th>Age (days)</th>
<th>Slope at high infusion rates (min)</th>
<th>Slope calculated at this rate interval (mg kg⁻¹ min⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allopregnanolone</td>
<td>14.0 (44.0)</td>
<td>4</td>
<td>C</td>
<td>70</td>
<td>0.82</td>
<td>4–8</td>
<td>This study</td>
</tr>
<tr>
<td>Allopregnanolone</td>
<td>7.9 (24.8)</td>
<td>3</td>
<td>A</td>
<td>60</td>
<td>ND</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Allopregnanolone</td>
<td>6.7 (21.0)</td>
<td>2</td>
<td>I</td>
<td>45</td>
<td>0.27</td>
<td>2–10</td>
<td>32</td>
</tr>
<tr>
<td>Allopregnanolone</td>
<td>5.1 (16.0)</td>
<td>2</td>
<td>I</td>
<td>110</td>
<td>ND</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Pregnanolone</td>
<td>10.5 (33.0)</td>
<td>4</td>
<td>C</td>
<td>70</td>
<td>0.57</td>
<td>4–8</td>
<td>This study</td>
</tr>
<tr>
<td>Pregnanolone</td>
<td>8.9 (27.9)</td>
<td>2</td>
<td>I</td>
<td>50</td>
<td>0.34</td>
<td>2–10</td>
<td>32</td>
</tr>
<tr>
<td>Pregnanolone</td>
<td>6.6 (20.7)</td>
<td>2</td>
<td>I</td>
<td>115</td>
<td>0.53</td>
<td>2–10</td>
<td>32</td>
</tr>
<tr>
<td>Barbiturates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexobarbital (Rac)</td>
<td>63.0 (243.0)</td>
<td>15</td>
<td>W</td>
<td>&gt;120</td>
<td>0.34</td>
<td>15–60</td>
<td>21</td>
</tr>
<tr>
<td>Hexobarbital (+)-S</td>
<td>46.2 (178.2)</td>
<td>15</td>
<td>W</td>
<td>280</td>
<td>ND</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>Hexobarbital (-)-R</td>
<td>148.3 (572.0)</td>
<td>15</td>
<td>W</td>
<td>280</td>
<td>ND</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>Narcobarbital (Rac)</td>
<td>72.8 (223.2)</td>
<td>15</td>
<td>W</td>
<td>&gt;65</td>
<td>0.61</td>
<td>15–30</td>
<td>34</td>
</tr>
<tr>
<td>Narcobarbital (+)</td>
<td>61.3 (187.9)</td>
<td>15</td>
<td>W</td>
<td>&gt;65</td>
<td>ND</td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>Narcobarbital (-)</td>
<td>84.5 (259.0)</td>
<td>15</td>
<td>W</td>
<td>&gt;65</td>
<td>ND</td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>Amyobarbital</td>
<td>71.0 (284.8)</td>
<td>10</td>
<td>W</td>
<td>&gt;120</td>
<td>1.10</td>
<td>10–40</td>
<td>21</td>
</tr>
<tr>
<td>Thiobutabarbitual</td>
<td>79.9 (317.9)</td>
<td>20</td>
<td>W</td>
<td>&gt;200</td>
<td>1.61</td>
<td>20–50</td>
<td>6</td>
</tr>
<tr>
<td>Pentobarbital (Rac)</td>
<td>56.1 (225.1)</td>
<td>5</td>
<td>W</td>
<td>&gt;120</td>
<td>1.07</td>
<td>5–15</td>
<td>21</td>
</tr>
<tr>
<td>Thiopental (Rac)</td>
<td>38.3 (144.9)</td>
<td>10</td>
<td>W</td>
<td>&gt;120</td>
<td>0.55</td>
<td>10–40</td>
<td>21</td>
</tr>
<tr>
<td>Methohexital (Rac)</td>
<td>13.9 (48.9)</td>
<td>10</td>
<td>W</td>
<td>&gt;120</td>
<td>0.11</td>
<td>10–30</td>
<td>21</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flurazepam</td>
<td>72.0 (185.6)</td>
<td>15</td>
<td>W</td>
<td>125</td>
<td>2.71</td>
<td>15–25</td>
<td>35</td>
</tr>
<tr>
<td>Propofol</td>
<td>13.4 (75.2)</td>
<td>10</td>
<td>I</td>
<td>90</td>
<td>0.34</td>
<td>10–20</td>
<td>36</td>
</tr>
<tr>
<td>Circulation time (tail to brain)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.10</td>
<td>–</td>
<td>21</td>
</tr>
</tbody>
</table>
indicates that the increased threshold dose of allopregnanolone and pregnanolone at low infusion rates could have been a result of the development of acute tolerance. The steroid infusion time required to reach SS was within the range that caused acute tolerance to hexobarbital.\(^6\) Analysis of hexobarbital concentrations showed an increased concentration in all brain areas studied as the infusion rate decreased,\(^6\) which suggested decreased brain sensitivity. Such an increased concentration could be used as a direct measure of acute tolerance.\(^4\)\(^1\) However, the development of acute tolerance in the allopregnanolone and pregnanolone groups was not supported by our results. Significantly lower steroid concentrations were found in cortex, striatum and cerebellum when the infusion rate was low. This is the reverse of what would have been expected if tolerance had developed. Thus, we conclude that acute tolerance did not develop to the anaesthetic effect during infusions of allopregnanolone and pregnanolone lasting 10 min or less.

Analysis of allopregnanolone and pregnanolone in all brain areas revealed that the brain had similar sensitivity to the two steroids. Despite the observation of a significant increase in steroid concentrations in cortex, striatum and cerebellum as the infusion rate was increased, the steroid concentrations in brainstem and hippocampus remained unchanged (Fig. 2). This finding indicated that anaesthetic sensitivity to allopregnanolone and pregnanolone in the brainstem and hippocampus was stable and independent of the changes in infusion rates and serum steroid concentrations. As we were using a threshold technique with a defined criterion recorded directly from the brain—a criterion that was reached with all dose rates and that was also used to terminate the infusion—this is the expected result. Our earlier results with hexobarbital indicated that critical brain areas with respect to induction of anaesthesia could be defined as areas with no change in active drug concentrations that depended on the infusion rate.\(^6\) Using this definition, cortex, striatum and cerebellum were excluded as the primary site of action with respect to allopregnanolone- or pregnanolone-induced anaesthesia. Thus, the remaining brain areas—the brainstem and hippocampus—may be considered as the primary sites of action for both steroids.

The brainstem was also distinguished as the only part of the brain that retained a relationship between threshold dose and tissue concentration. For both steroids, the relationship was recorded as a weak but significant correlation (Fig. 3A and C). Because the threshold technique depends on a simple feedback system, a strong correlation founded on a direct relationship is to be expected from a methodological point of view. However a comparison of the dose rate curves (Fig. 1A) with the corresponding serum concentrations (Fig. 1B) points to complex pharmacokinetics. When the infusion rate is increased, higher serum concentrations are needed to reach the concentration in the brainstem found at SS. This is best illustrated by the quotient between concentrations in the brainstem and serum. With allopregnanolone the quotient was 0.78 (0.13) at the infusion rate of 2.0 mg kg\(^{-1}\) min\(^{-1}\) and 0.30 (0.04) at the rate of 8.0 mg kg\(^{-1}\) min\(^{-1}\). The corresponding values with pregnanolone were 0.82 (0.07) and 0.45 (0.05). Despite this altered penetration, a weak correlation between threshold dose and tissue concentration was still present when the brainstem was the target organ (Fig. 3A and C). Instead of a direct, strong relationship, only around 30% of the variability of the concentration in the brainstem was explained by the dose, but as it was the only correlation found in the parts of the brain that were analysed, this strengthens the possibility that the brainstem has a critical function in the induction of the SS.

In the present study, a linear correlation between threshold dose and the concentration in fat at SS was observed for both allopregnanolone and pregnanolone (Fig. 3B and D). No significant correlation between threshold dose and muscle concentration was observed. When we studied the tissue concentrations in different compartments, we found that the steroid concentrations in serum and muscle were significantly correlated for both allopregnanolone and pregnanolone, while the correlation between serum and fat was not significant for any of the steroids. This suggests that the muscle compartment was involved in the rapid distribution of steroid. However, fat could be regarded as a special tissue that actively processes allopregnanolone and pregnanolone in a similar way to the brainstem, because a significant linear correlation between threshold dose and tissue concentration of both steroids was found only for brainstem and fat (Fig. 3).

As discussed by Bolander et al.,\(^2\) the threshold dose at the optimal dose rate could be used as a comparable measure of the potency of allopregnanolone and pregnanolone in adult animals. When the threshold doses of two steroid anaesthetics were compared, we found that the threshold doses of allopregnanolone were significantly higher than the corresponding threshold doses of pregnanolone at SS. This was in variance with a previous study using Intralipid\(^{3\text{a}}\) as the steroid solvent, in which the average threshold dose for allopregnanolone was significantly lower than that of pregnanolone at SS in both young and adult rats.\(^3\)\(^2\) However, serum concentrations of allopregnanolone and pregnanolone in the present study were not significantly different from each other (Fig. 1). Since the anaesthetic sensitivity in rat brainstem and hippocampus remained stable as the infusion rate was increased, we tentatively considered these two brain areas as the primary sites of action and compared the steroid concentrations in the two brain areas. Allopregnanolone concentrations in the brainstem and hippocampus were generally lower than those of pregnanolone (Table 1), suggesting that allopregnanolone was more potent than pregnanolone in inducing anaesthesia. Results from the present study indicate that the active drug concentrations at the primary sites of action are in agreement with previous findings.\(^3\)\(^3\) Our different results with regard to threshold doses could have been due to the
steroid solvent we used, β-cyclodextrin in place of Intralipid®. This indicates that i.v. cyclodextrin solution could alter the pharmacokinetic properties and clinical behaviour of concurrently administered CNS depressant agents, and results obtained with cyclodextrin as a solvent must be evaluated carefully.

Acknowledgements
We thank Mrs K. Wahlström and Mrs. L. Gustavsson for their skilful technical assistance. This study was supported by Medical Research Council project 4X-11198, a 'Spjutsspets' grant from Umeå Sjukvårds och Systembolagets Fond.

References
7 Wahlström G, Bolander H. Dynamic aspects of acute tolerance to hexobarbital evaluated with anaesthesia threshold. Alcohol 1985; 2: 297–301
9 Mellanby E. Alcoholic: its absorption into and disappearance from the blood under different conditions. Special Report Series, No. 31. London: Medical Research Committee, 1919
13 Sinclair JG, Lo GF. Acute tolerance to ethanol on the release of acetylcholine from the cat cerebral cortex. Can J Physiol Pharmacol 1978; 56: 668–70
15 Smith RA, Winter PM, Smith M, Eger EI II. Rapidly developing tolerance to acute exposure to anaesthetic agents. Anesthesiology 1979; 50: 496–500
17 Li TK, Lumeng L, McBride WJ, Murphy JM. Rodent lines selected for factors affecting alcohol consumption. Alcohol 1987 (Suppl. 1): 91–6
18 Allan AM, Harris RA. Acute and chronic ethanol treatments alter GABA receptor-operated chloride channels. Pharmacol Biochem Behav 1987; 27: 665–70
27 Sundström I, Andersson A, Nyberg S, Ashbrook D, Purdy RH, Bäckström T. Patients with premenstrual syndrome have a different sensitivity to a neuroactive steroid during the menstrual cycle compared to control subjects. Neuroendocrinology 1998; 67: 126–38
30 Harrison NL, Simmonds MA. Modulation of the GABA_A receptor complex by a steroid anaesthetic. Brain Res 1984; 323: 287–92
33 Wahlström G. The interaction between atropine and the steric isomers of hexobarbital in normal rats and rats made tolerant to barbital. Eur J Pharmacol 1979; 59: 219–25
34 Wahlström G. Differences in anaesthetic properties between the optical isomers of 5-(2-bromoallyl)-5-isopropyl-1-methylbutyric acid (Enibomal NFN) in the rat. Acta Pharmacol Toxicol (Copenh) 1968; 26: 81–91
35 Norberg L, Wahlström G. Anaesthetic effects of flurazepam
alone and in combination with thiopental or hexobarbital evaluated with an EEG-threshold method in male rats. Arch Int Pharmacodyn Ther 1988; 292: 45–57