Dynamic aspects of acute tolerance to allopregnanolone evaluated using anaesthesia threshold in male rats

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Background. It is unclear if allopregnanolone (AlloP) anaesthesia can induce tolerance. Acute tolerance is defined as altered sensitivity to a drug during a single continuous exposure.

Methods. Induction of acute tolerance to AlloP was studied in male rats using a threshold technique of deep anaesthesia. AlloP was infused at a dose rate of 4.0 mg kg\(^{-1}\) min\(^{-1}\). The infusion was stopped when a burst suppression of 1 s or more (the ‘silent second’, SS) occurred in the EEG. To maintain anaesthesia, the infusion was restarted when no SS had been seen in the EEG for 1 min. This interrupted targeted infusion towards an EEG end-point (SS) was continued until 30, 60 or 90 min of anaesthesia had been reached. At these times the rats were killed and AlloP concentrations in serum, muscle, fat and different brain regions were determined by radioimmunoassay.

Results. Maintenance dose rate (MDR) was calculated using 20-min intervals. During anaesthesia the MDR increased (\(P<0.001\)) from 0.67 (SEM 0.03) mg kg\(^{-1}\) min\(^{-1}\) (in the interval 10–30 min) to 0.98 (0.04) mg kg\(^{-1}\) min\(^{-1}\) (in the interval 65–85 min). After 60 min a slight increase in MDR was observed. After 90 min of anaesthesia the AlloP concentrations in the hippocampus and brainstem had increased by more than 50% compared with control values of 25.2 (1.13) and 52.7 (5.81) nmol g\(^{-1}\) respectively, and after 60 min to around 40%. At 30 min no increase was seen in any brain region analysed.

Conclusions. Measurements in vivo and in vitro record acute tolerance to AlloP occurring with a delay.

Br J Anaesth 2004; 93: 560–7

Keywords: anaesthesia, depth; anaesthetics i.v., allopregnanolone; measurement techniques, radioimmunoassay; model, rat; pharmacology, tolerance

Accepted for publication: May 27, 2004
study we reassess the importance of the duration of development of anaesthesia in acute tolerance to AlloP by testing anaesthesia periods of 30–90 min.

Methods

Animals

Fifty male Sprague–Dawley rats (Mol:SPRD Han; M&B, Ry, Denmark) were used in two experiments. They were housed three to each cage in an animal room without external light. The temperature in the room was around 22°C and it had a reversed light–dark cycle (lights on between 19:00 and 07:00 h). The rats were allowed free access to water and standard food. After 1 week of acclimatization, the rats were weighed for age determination using an age–weight curve obtained from the breeder. In experiment 1 we used 20 rats. They had a mean weight of 319 (SEM 2.4) g, which gave an age of 62 days. In experiment 2 we used 30 rats. They had a mean weight of 348 (2.5) g which indicated an age of 65 days. The experiments were performed approximately 20 days after age determination. The experimental protocols were approved by the Regional Ethical Committee for Animal Experiments in Umeå (Umeå djurförsöketiska nämnd).

Drugs

Unless otherwise stated, chemicals used were of analytical quality and purchased from a local supplier. AlloP was purchased from Umecrine (Umeå, Sweden). AlloP was dissolved in 10% 2-hydroxypropyl-β-cyclodextrin (β-cyclodextrin; Sigma, St Louis, MO, USA) at a concentration of 3 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 under visual inspection.

EEG threshold method

The anaesthetic effect of AlloP in rats was determined with an i.v. EEG threshold method. The AlloP solution was infused i.v. with a Sage Instruments Model 355 syringe pump (Orion Research, Cambridge, MA, USA) via a tail vein with a constant, optimal infusion rate of 4 mg kg⁻¹ min⁻¹. This optimal dose rate was defined as that which gave the lowest threshold dose when different dose rates were tested. Differences in body weight were compensated by small corresponding changes in infusion rate. The EEG was recorded continuously with a Mingograph EEG 10 (Siemens-Elema, Stockholm, Sweden), from subcutaneous stainless steel electrodes placed in a bifrontal configuration with a crocodile clip attached to one of the ears as a signal ground. This crocodile clip was not attached until the rat was under deep anaesthesia. The infusion was immediately stopped when the first burst suppression period of 1 s or more was recorded in the EEG (the ‘silent second’, SS). As illustrated in an earlier paper, the SS is easily detected against a background of an EEG, which at this stage consists mainly of high-amplitude potentials. The appearance of SS occurs at a deeper level of anaesthesia than the loss of righting reflex. The time to reach SS was recorded, and the amount of AlloP needed to induce the effect was calculated. This dose was considered to be the threshold dose. The threshold doses recorded in all groups are given in Fig. 1A.

Experimental design

The rats were randomly assigned to the order in which they were tested and all EEG testing was done between 08:30 and 14:30. In the experimental groups anaesthesia was maintained for different durations of time after induction of the first SS. At the first SS the infusion was stopped, but the EEG recording continued. At this SS there is some AlloP which has not yet reached the site of action. This overdose will give a duration of the SS after the infusion is stopped. The last SS during such a sequence of burst suppression in the present experiments usually occurred approximately 1 min after the end of infusion. After the last SS in a sequence one more minute was allowed before the infusion was started again. At the next SS the infusion was stopped a second time and a new target controlled infusion started. When the predetermined duration of anaesthesia in an individual rat was close, it was decided whether the next infusion should be the last. Since all rats were killed at the first SS during the last infusion, this gives slight uncertainty in the duration of anaesthesia. This experimental design has been described in detail earlier. During long periods of anaesthesia, rectal temperature was measured and kept at the initial level by regulating the distance to an external heat source. Heart rate, recorded from the EEG during the periods of SS after each infusion, and respiratory rate, measured by counting, were both reasonably stable during long periods of anaesthesia.

Since the interval between infusions during the long periods of anaesthesia varied between individual rats, cumulative doses were calculated with intervals of 5 min (Fig. 1B–D). These cumulative doses were used to calculate MDRs (mg kg⁻¹ min⁻¹) where differences in cumulative doses between two time points in individual rats were divided by the time interval used. In the present experiments a 20-min interval was used since several infusions had been performed during that time, which gave an adequate certainty.

When we designed the present study, we performed a pilot study with an anaesthesia duration of 60 min. This pilot study is presented as experiment 1. A control group of nine rats were killed at the first SS. One rat was excluded because of technical difficulties. Group 60 min, in which the rats were killed after 60 min of anaesthesia, consisted of eight rats. Two rats were excluded because of technical
difficulties. After dissection, the AlloP concentrations were determined in different parts of the brain.

The pilot study was followed up in experiment 2, in which the effects of 30 and 90 min of anaesthesia were investigated. A control group of nine rats were killed at the first SS, in which one rat was excluded because of technical difficulties. Group 30 min, in which anaesthesia lasted 30 min, consisted of nine rats. Group 90 min, in which anaesthesia lasted 90 min consisted of nine rats. Two rats were excluded from the last two groups because of subcutaneous infusions. After dissection, only the right half of the brain was used to determine AlloP concentrations.

**Tissue sample preparation**

The rats were killed by decapitation immediately at the end of the predetermined last infusion. Trunk blood was collected and the brain was dissected immediately into the following parts: cerebellum, cortex, hippocampus, brainstem (medulla oblongata and midbrain) and striatum, largely according to Glowinski and Iversen.\(^{12}\) The major blood vessels on the surface of the brain hemispheres were carefully removed when dissecting the brain. A macroscopic autopsy was always carried out. Abdominal fat tissue from the retroperitoneal area and part of the psoas muscle tissue were removed from each rat. After weighing, the tissue was frozen at \(-70^\circ\text{C}\) until analysis. All the samples were extracted later with 99.5% ethanol for 7 days at \(+4^\circ\text{C}\). The recovery of steroids in this procedure has previously been shown to be 100%.\(^8\)

**Celite chromatography and steroid assay**

AlloP in tissue and serum extracts were separated with celite chromatography, as described by Bäckström and colleagues\(^7\) and verified by Corpéchot and colleagues.\(^7\) The recovery rate of AlloP from celite chromatography was determined in every assay using the \(^{3}\text{H}\)allopregnanolone tracer as an indicator of recovery. Recovery of AlloP was 85%. The concentration of AlloP in brain tissue extracts was

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**Fig 1** Doses of allopregnanolone recorded during threshold testing. (a) Threshold doses at the first silent second (SS) in each group of experiments 1 and 2. (b) Cumulative doses in group 60 min in experiment 1. (c) Cumulative doses in group 30 min in experiment 2. (d) Cumulative doses in group 90 min in experiment 2.
measured by radioimmunoassay. Radioactive steroid tracer [9,11,12-\textsuperscript{3}H(N)]-5α-pregnan-3α-ol-20-one was purchased from NEN Life Science Products (Boston, MA, USA). AlloP antiserum was raised against 3α-hydroxy-20-oxo-5α-pregn-11α-yl carboxymethyl ether coupled to bovine serum albumin. The antiserum was kindly provided by Dr Robert Purdy (San Diego, CA, USA). Cross-reactivity has been tested earlier\textsuperscript{26,30} and was highly specific. The sensitivity of both assays was 25 pg, with an intra-assay coefficient of variation of 7% and inter-assay coefficient of variation of 8%. The intra-assay coefficient of variation is calculated from duplicate values in the assay.

**Data analysis**

A two-way ANOVA, followed ad hoc by a least significant difference test, was used to test the significance of differences for possible pairs in all series. When assessing the maintenance dose rates at different time intervals, the significance of differences was tested with paired Student’s \( t \)-test for independent samples. Linear parametric correlations (\( r \)) and regression coefficients (\( b \)) were calculated with conventional methods. All statistical calculations were performed using SPSS statistical software (Chicago, IL, USA). A value of \( P<0.05 \) in the two-tailed test was taken to represent significant differences. Non-significant differences are indicated by ‘NS’. All results are presented as means with one standard error of mean (SEM); \( n \) denotes the number of animals in each test and d.f. is the number of degrees of freedom.

**Results**

**In vivo measurements**

Initial CNS sensitivity, in terms of threshold dose of AlloP at the first SS, is shown in Fig. 1A. There was no significant difference in initial sensitivity between individual groups within either experiment (NS, two-way ANOVA). The present experimental method was used to maintain anaesthesia in order to measure changes in sensitivity over time. Data from experiment 1 (Fig. 1B) and experiment 2 (Fig. 1C, D) are shown as the dose of AlloP needed to maintain anaesthesia (cumulative dose) up to the time of killing, plotted against the duration of anaesthesia. It is evident from Fig. 1B–D that there was a steady non-linear increase in cumulative dose with respect to anaesthesia time.

The properties of the present system are further evaluated in Figure 2 by using the calculated dose rate of AlloP needed to maintain anaesthesia (MDR, mg kg\(^{-1}\) min\(^{-1}\)). The difference in cumulative dose determined at 20-min intervals revealed a significant increase in MDR with regard to anaesthesia time in both experiments (paired \( t \)-test). In group 60 min of experiment 1 the MDR at the beginning of anaesthesia (MDR\(_{10–30}\) min=0.70 (0.03) mg kg\(^{-1}\) min\(^{-1}\)) was significantly different from that at the end of anaesthesia (MDR\(_{35–55}\) min=0.79 (0.04) mg kg\(^{-1}\) min\(^{-1}\); \( t=4.27, \) d.f.=7, \( P<0.01 \)). In group 90 min of experiment 2 the MDR at the beginning of anaesthesia (MDR\(_{10–30}\) min=0.67 (0.03) mg kg\(^{-1}\) min\(^{-1}\)) was not significantly different from that in the middle of anaesthesia (MDR\(_{35–55}\) min=0.78 (0.04) mg kg\(^{-1}\) min\(^{-1}\); \( t=1.96, \) d.f.=8, \( P<0.10 \)), while the MDR at the end of anaesthesia (MDR\(_{65–85}\) min=0.98 (0.04) mg kg\(^{-1}\) min\(^{-1}\)) was significantly increased when compared with MDR\(_{10–30}\) min and MDR\(_{35–55}\) min (\( t=9.42, \) df=8, \( P<0.001 \) and \( t=3.56, \) df=8, \( P<0.01 \), respectively). Furthermore, in experiment 2 there was no significant difference between the MDR\(_{10–30}\) in the group which had only 30 min of anaesthesia and the corresponding MDR in the group which had had 90 min of anaesthesia. Thus it is clear that acute tolerance, recorded as the increase in threshold dose needed to maintain SS, is evident after 90 min of anaesthesia. The induction of acute tolerance can also be traced in vivo after 60 min of anaesthesia.

**In vitro measurements**

Figures 3 and 4 demonstrate the complete data on the concentrations of AlloP at the SS in serum, different brain regions and fat and muscle tissues in the different groups from experiment 1 and experiment 2. After 30 and 60 min of anaesthesia in both experiments, the AlloP concentrations in serum remained stable at about 40 μM, which is the concentration recorded in the control groups (Fig. 3). After 90 min of anaesthesia, the serum concentration of AlloP increased significantly to a concentration around 60 μM (Fig. 3). The AlloP concentrations in muscle tissue...
remained stable in all groups regardless of the duration of anaesthesia (Fig. 3). We observed a significant increase in AlloP concentrations in fat that were related to the duration of anaesthesia when compared with the respective control groups (Fig. 3). It is evident that there is an active process of redistribution of AlloP to the fat compartment as the duration of anaesthesia is increased. Such a redistribution was seen already after 30 min of anaesthesia, and the mean values for the different groups were very close to linearity when analysed against time in anaesthesia ($r=0.99$, $b=1.17$, d.f.=3, $P<0.01$, Fig. 5).

In experiment 1, measurements of AlloP in different brain regions after 60 min of anaesthesia revealed a significant increase in the hippocampus (+42%) and brainstem (+38%) compared with the concentrations at the first SS in the control group (Fig. 4). In this experiment, there was no corresponding increase in serum concentration (Fig. 3). In experiment 2 (Fig. 4), there was a significant increase in AlloP concentration after 90 min of anaesthesia in the hippocampus (+58%) and brainstem (+51%). However, in this experiment, there was also a corresponding increase in serum concentration (Fig. 3). In experiment 1 and experiment 2, the concentrations of AlloP in the striatum, cerebellum and cortex remained stable when plotted against the duration of anaesthesia (Fig. 4), regardless of the increase in serum concentration seen in experiment 2 (Fig. 3). We have shown earlier that the hippocampus and the brainstem might be the primary sites of action with respect to AlloP-induced anaesthesia. Thus, acute tolerance (seen as increases in the concentrations of AlloP in critical brain regions) is recorded as the duration of anaesthesia is prolonged. The induction can be measured after 60 min of anaesthesia, but no acute tolerance is seen after 30 min of anaesthesia.

**Relationship between in vivo and in vitro measurements**

In experiment 2 there were some interesting correlations between variables from the group killed after 30 min of anaesthesia. Both MDR$_{10-30}$ min and AlloP concentrations in the hippocampus were positively correlated with the dose of AlloP required to induce the first SS ($r=0.78$, $n=9$, $b=0.04$, $P<0.05$ and $r=0.81$, $b=1.53$, $n=9$, $P<0.01$, respectively).
Increased in AlloP in the hippocampus (of anaesthesia, brain concentrations showed a significant increase in AlloP sensitivity measured in the same rats, which indicates that no change had occurred.

Acute and chronic tolerance15 can result either from an increased disappearance rate of the drug from the site of action (dispositional tolerance) or from decreased sensitivity at the site of action (functional tolerance). If the MDR measurements are combined with measurements of drug concentrations in different tissues after the first induction and after different durations of anaesthesia, it is possible to distinguish between the two. Because of the increased concentration found in the hippocampus and brainstem after 90 min of anaesthesia (Fig. 4), it is possible to state that functional tolerance to AlloP had been induced in the present experiments.

In interpreting experiments on acute tolerance, it is important to investigate the influence of the duration of exposure to the drug. In the present experiments the development of acute tolerance to AlloP can be determined by changes in drug concentration and measurements of MDR. No sign of tolerance development was obtained after 30 min anaesthesia, as the concentrations of AlloP in the brain were not changed compared with the concentrations found at induction. Furthermore, a high correlation was noted between MDR10–30 min and the threshold dose of AlloP at induction in the same rats, which indicates that no change had occurred in AlloP sensitivity measured in vivo at 30 min. After 60 min of anaesthesia, brain concentrations showed a significant increase in AlloP in the hippocampus (+42%) and brainstem (+38%). The corresponding MDR10–30 min increased by only 13%, but was significantly different from the MDR10–30 min.

After 60 min of anaesthesia in experiment 2, there was no corresponding significant difference between MDR35–55 min and MDR10–30 min. Thus, the AlloP tissue concentrations at 60 min gave a clearer indication of acute tolerance than the MDR35–55 min. This suggests that the changes in AlloP concentrations had occurred close to the end of 60 min of anaesthesia. After 90 min of anaesthesia, well-defined acute tolerance was recorded both in vitro and in vivo. Consequently, these results show that functional acute tolerance to AlloP develops, but its expression seems to be delayed to around 60 min of anaesthesia.

The AlloP concentrations in the hippocampus and brainstem increased significantly with duration of anaesthesia. In the cortex, cerebellum and striatum the concentration remained unchanged when the duration of anaesthesia was prolonged. This indicates that of the brain regions investigated, the hippocampus and brainstem seem to be the most interesting in relation to the induction of acute tolerance. This conclusion is supported by parallel changes in in vitro concentrations and in vivo measurements of MDR at the different durations of anaesthesia. Earlier results also indicate that the hippocampus and brainstem are involved in the induction of anaesthesia with AlloP.36 The effect of different dose rates on the threshold doses and corresponding brain concentrations of AlloP was investigated. At the anaesthesia criterion, the concentrations of AlloP should, in critical brain regions, remain unchanged despite changes in dose rate, and this occurred in the hippocampus and brainstem but not in serum, cortex, striatum, cerebellum and muscle.36 In addition, there are other studies indicating that AlloP acts in the hippocampus. AlloP is known to decrease excitability in hippocampal slices at very low concentrations.34 Intraperitoneal administration of AlloP at doses regarded as medium (15 mg kg\(^{-1}\)) and high (17.5 mg kg\(^{-1}\)) produced significant reductions in the spontaneous firing rate of hippocampal pyramidal neurons in adult male rats, which lasted at least 60 min. Low doses (10 mg kg\(^{-1}\)) had no effect.31 Recent findings14 that AlloP treatment degrades spatial learning in rats tested in the Morris water maze also point to the hippocampus. A latency of 8 min between AlloP (2 mg kg\(^{-1}\)) injection and testing gave a positive response but the corresponding test with a latency of 20 min had no effect. Furthermore, the hippocampus was the region of the brain where the largest difference in AlloP concentration between these two test occasions was found.14 Significant increases in progesterone and pregnandione concentrations in the hippocampus were noted in progesterone-induced anaesthesia, and it was apparent that more pronounced metabolism of progesterone occurs in the hippocampus than in other brain regions.2 In mice, however, progesterone-reduced muscimol binding in the nucleus accumbens and the nucleus caudatus caused a marginal change in the gyrus dentatus but no changes in CA3 of the hippocampus and three regions of the cortex. Because these changes occurred in the GABA\(_A\) system, they were probably mainly induced by the metabolite AlloP.8 Information on effects in which
the brainstem is involved is less abundant. Frye and Vongher\textsuperscript{11} showed that local injections of three AlloP synthesis inhibitors into the ventral tegmental area reduced the concentration of AlloP and also reduced the lordosis response in hormone-primed females. These responses seemed to be specific, as no similar responses were obtained in other midbrain areas tested.

A comparison of serum and brain concentrations of AlloP indicates a specific kinetic situation in the present experiments. At 60 min (Figs 3 and 4) the concentration in the hippocampus and brainstem had increased without a primary increase in the serum. No similar increase was found in the other brain regions. At 90 min (Figs 3 and 4) the increases in the hippocampal and brainstem concentrations were slightly larger, but there was a corresponding increase in the serum concentration. In this case there were no changes in concentrations in the other brain regions. These results indicate that specific uptake is involved in the distribution of AlloP in the hippocampus and brainstem.

The mechanism of this specific uptake was not investigated in the present work and we can therefore only speculate about the reasons. However, differences in uptake could result from a high number of high-affinity AlloP-binding sites in the hippocampus and brainstem, increased concentrations in the cell membrane or active transport from the blood to these regions. One hypothesis is that AlloP binds to a high-affinity binding site on the GABA\textsubscript{A} receptor.\textsuperscript{24} No such binding site has so far been identified. However, different studies demonstrate that the interaction of AlloP with the GABA\textsubscript{A} receptor is dependent on the subunit composition and that the subunit composition varies between brain regions. In addition, there are changes in the subunit composition caused by AlloP exposure. Increased binding of AlloP to a modified GABA\textsubscript{A} receptor could explain the increased concentrations of AlloP in the hippocampus and brainstem described in the present paper. The delay of around 60 min in the induction of acute tolerance to AlloP could be long enough for the reorganization of the GABA\textsubscript{A} receptors in some specific brain regions. The possibility that neurenes regulate the postsynaptic GABA\textsubscript{A} receptors by trafficking is another way to account for the neuronal changes involved in the development of the acute tolerance.\textsuperscript{16} These issues certainly merit further investigation.

An alternative explanation for the increased AlloP concentration in the hippocampus and brainstem could be high lipid solubility of AlloP. Increased uptake in the membrane could depend on the fluidity of the plasma membrane. This is a well-known explanation of the anesthetic activity of lipophilic drugs;\textsuperscript{13,23} it has been questioned but not refuted. This possibility cannot be disregarded when dealing with AlloP, as it has been shown in neurenes from the hippocampus that cholesterol enrichment of the neuronal membrane can reduce the effect of AlloP on GABA-induced currents.\textsuperscript{29} This could decrease the potency of AlloP on the GABA\textsubscript{A} receptor but so far it cannot explain the specific increases in AlloP concentrations in the hippocampus and brainstem.

Data on the concentrations of AlloP in fat and muscle show that redistribution to fat, but not to muscle, is the important component in the elimination of AlloP from the brain. After 90 min of anaesthesia, data (Fig. 5) show that there was still no limit to the capacity of fat to accumulate AlloP and also that there was no increase in redistribution. Thus, it is unlikely that the high concentrations obtained in the hippocampus and brainstem can be explained by decreased transport out of relevant cells. Turnover cannot be determined, but a substantial amount of AlloP must have accumulated in the fairly large fat depot, indicating that the AlloP in the brain was easily accessible to the blood when the concentration in serum was reduced during the intervals between infusions. This supports the hypothesis that AlloP in the brain is bound to sites with affinity that is relatively low but higher than that existing in blood, as accumulation compared with blood concentrations had occurred. The increase in AlloP in the hippocampus and brainstem may thus be due to increased binding at some neurosteroid sites on the GABA\textsubscript{A} receptor, where changes in receptor structure and/or number could determine binding capacity.

In earlier experiments using the anaesthesia threshold method, acute tolerance to hexobarbital\textsuperscript{12,33} and propofol\textsuperscript{20} was recorded with the threshold technique. With hexobarbital, maximum acute tolerance had been established after 60 min, seen as a 35–45% increase in concentrations in serum, cortex and brainstem. These rats were older than those used in the present experiment. With propofol, 60 min of anaesthesia did not induce any measurable acute tolerance in 30-day-old rats, but in 460-day-old rats acute tolerance with significantly increased concentrations (20–50%) were recorded in serum, hippocampus, striatum, brainstem and cerebellum. No increase was seen in the cortex. A comparison with the present results using AlloP indicates that age could be a critical factor. However, there might also be specificity in the brain regions involved in the induction of acute tolerance by different anaesthetic agents. This needs further investigation.

Acknowledgements
We thank Mrs K. Wahlström and Mrs L. Gustafsson for their skilled technical assistance, and the help received from Dr Inga-Maj Johansson is gratefully acknowledged. This study was supported by the Medical Research Council project No. 4X-11198, a ’Spjutspets’ grant from Umeå Sjukvärd, an EU regional fund Objective 1 program grant and grants from the Faculty of Medicine and Dentistry, Umeå University, ‘Folkhälsoinstitutet’ and ‘Systembolagets forskningsfond’.

References


6 Brodie BB, Mark LC, Leif PA, Bernstein E, Papper EM. Acute tolerance to hexobarbital evaluated with anaesthesia threshold. *Br J Anaesth* 2001; 86: 53–79


18 Korkmaz S, Wahlstrom G. The EEG burst suppression threshold test for the determination of CNS sensitivity to intravenous anaesthetics in rats. *Brain Res Protoc* 1997; 1: 378–84


27 Reddy DS, Rogawski MA. Chronic treatment with the neuroactive steroid ganaxolone in the rat induces anticonvulsant tolerance to diazepam but not to itself. *J Pharmacol Exp Ther* 2000; 295: 1241–8


29 Sookswate T, Simmonds MA. Increased membrane cholesterol reduces the potentiation of GABA<sub>A</sub> currents by neurosteroids in dissociated hippocampal neurones. *Neuropharmacology* 1998; 37: 1103–10


33 Wahlström G, Bolander HG. Dynamic aspects on acute tolerance to hexobarbital evaluated with anaesthesia threshold. *Alcohol* 1985; 2: 297–301

