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Tolerance to the anticonvulsant activity of midazolam and allopregnanolone in a model of picrotoxin seizures

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Abstract

The effects of an intracerebroventricular (i.c.v.) administration of a non-selective full benzodiazepine receptor agonist, midazolam, and a neuroactive steroid, allopregnanolone, on picrotoxin-induced seizures and striatal dopamine metabolism, were studied in mice. It was found that acute i.c.v. injections of midazolam (ED_{50} = 38.25 nmol) and allopregnanolone (ED_{50} = 26.34 nmol) blocked picrotoxin-induced seizures to a similar extent. After repeated administration at the ED_{85} doses (midazolam—56.6 nmol, allopregnanolone—94.2 nmol; once or twice daily for 5 days) tolerance developed to the anticonvulsant activity of midazolam (ED_{50} = 94.14 nmol) and allopregnanolone (ED_{50} = 186.70 nmol). Acute i.c.v. injections of midazolam and allopregnanolone (at the ED_{50} doses established in the model of picrotoxin seizures: 38.25 and 26.34 nmol, respectively), significantly decreased the concentration of dopamine metabolites: 3-methoxytyramine and 3,4-dihydroxyphenylacetic acid, as well as the dopamine turnover rate (homovanilic acid/dopamine ratio; by about 20%), in the mouse striatum. These findings together with the recently published data on the potentiation by midazolam and allopregnanolone of ethanol-induced sleep [Pharmacol. Biochem. Behav. 67 (2000) 345] indicate a very similar central effect profile of benzodiazepines and neurosteroids. Moreover, similar efficacy of allopregnanolone and midazolam at the $GABA_A$ receptors has been found. Overall, the results of the present study, along with the possibility of neurosteroid conversion in the brain into other steroid hormones (testosterone, estradiol, aldosterone), add to the accumulating evidence suggesting a less favorable pharmacological profile for this class of drugs than was previously thought. © 2001 Published by Elsevier Science B.V.

Keywords: Picrotoxin-induced seizure; Midazolam; Allopregnanolone; Intra cerebroventricular injection; Dopamine turnover rate; Striatum; (Mouse)

1. Introduction

Neurosteroids are potent and specific endogenous modulators of $GABA_A$ receptors, which regulate many brain functions. Recently, some new compounds with improved oral bioavailability and reduced metabolic liability have been developed (Wieland et al., 1997; Gąsior et al., 1999, 2000; Reddy and Rogawski, 2000a; Vanover et al., 2000). These derivatives of naturally occurring metabolites of steroid hormones are currently considered as having a future role in the management of epilepsy, anxiety, insomnia, migraine and drug dependence (Gąsior et al., 1999). However, certain obstacles still hamper the clinical use of analogs of endogenously occurring neurosteroids. For example, their anticonvulsant activity must be maintained with chronic dosing.

Although it is now well established that neuroactive steroids produce many central effects on acute administration, there is conflicting information regarding the effects of neuroactive steroids when administered repeatedly. On the one hand, there are experimental data indicating that tolerance develops rapidly to the central actions of neurosteroids. For example, chronic dosing with minaxolone [(2β, 3α, 5α, 11α)-2-ethoxy-3-hydroxy-11,*N*,*N*-dimethyl-

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amino-pregnane-20-one] once daily for 7 days results in a loss of sedative response to an acute dose of the drug (Marshall et al., 1997).

On the other hand, chronic treatment with neurosteroids which are negative modulators of the $GABA_A$ receptor complex (pregnenolone sulfate and dehydroepiandosterone sulfate), significantly shifted the pentylenetetrazol dose-percent convulsion and latency curves to the left, and markedly decreased the ED_{50} of pentylenetetrazol for tonic convulsions, indicating an increased sensitivity of mice to seizures (Reddy and Kulkarni, 1998). Chronic neurosteroid treatment also decreased the efficacy of benzodiazepine ligands and neurosteroids at the $GABA_A$ receptor complex in cortical neurons (γ-aminobutyric acid-induced $^{36}Cl^-$ influx in intact cultured neurons) (Yu and Ticku, 1995a,b). Moreover, withdrawal from chronic progesterone treatment attenuates the behavioral response (sedative effect in the treadmill locomotion test) to lorazepam as a direct result of increases in the α4 subunit of the $GABA_A$ receptor (Moran et al., 1998).

These findings indicate a possible mechanism of tolerance to the central effects of neurosteroids. Collectively, these results suggest that long-term administration of neurosteroids, like that of benzodiazepines, brings about adaptive changes in the $GABA_A$ receptor complex functions, thus probably limiting their clinical utility.

Several recently published results suggest that tolerance does not develop to the anticonvulsant activity of pregnanolone (Kokate et al., 1998), and ganaxolone (a 3β-methylated analog of the naturally occurring neurosteroid allopregnanolone) (Gąsior et al., 1999, 2000; Reddy and Rogawski, 2000a,b), in mice, as well as to the anxiolytic-like action of an analog of ganaxolone, Co 3-0593 (3β-ethenyl-3α-hydroxy-5α-pregnan-20-one), in the Geller–Seifter test (Wieland et al., 1997), with rats. Furthermore, whereas the ED_{50} values for ganaxolone in the pentylenetetrazol seizure test after 3- and 7-day treatment with ganaxolone were not different from those in naive rats, there was a significant reduction in the anticonvulsant potency of diazepam in these animals (Reddy and Rogawski, 2000b). These data indicate that chronic ganaxolone treatment led to cross-tolerance to diazepam but not to itself.

Recently, a novel neuroactive steroid, Co 2-6749 (3α, 21-dihydroxy-3β-trifluoromethyl-19-nor-5β-pregnan-20-one), has been shown to posses robust anxiolytic-like effects across species (rats and pigeons), a wide separation between anxiolytic-like effects and sedation/ataxia, a minimal interaction with ethanol, and a lack of tolerance to anxiolytic activity (Vanover et al., 2000). Moreover, in the model of finasteride-induced reduction of the threshold for pentylenetetrazol seizures, in a state of persistently high serum progesterone (pseudopregnancy), there was a three-fold increase in the anticonvulsant potency of ganaxolone, and a decrease in the anticonvulsant potency of diazepam (Reddy and Rogawski, 2000a). It was also shown that there were no substantial pharmacokinetic changes in the half-life, and brain and plasma levels of pregnanolone and ganaxolone during their prolonged administration, which could account for their central effects (Kokate et al., 1998; Reddy and Rogawski, 2000b).

It appears, therefore, that the problem of adaptive changes in the anticonvulsant activity of neuroactive steroids occurring during repeated administration, and their interaction with other neurotransmitter systems, is not well understood. The limited experimental data available are not homogeneous, as they have been obtained mostly after peripheral administration of drugs which can be metabolized to other steroids with different receptor profiles (Baulieu, 1998). Therefore, we have decided to examine this problem further using mice injected repeatedly intracerebroventricularly (i.c.v.) with allopregnanolone and midazolam, in the model of picrotoxin-induced convulsions. In this way, the acute and chronic effects of a full non-selective allosteric modulator of the $GABA_A$ receptor complex, and the most potent neurosteroid, could be compared directly.

In addition, as benzodiazepines and neurosteroids were shown to produce similar side-effects, including sedation and motor impairments, i.e. phenomena related to dopaminergic system function, the influence of acute i.c.v. injections of midazolam and allopregnanolone on the striatal metabolism of dopamine was also examined. Such pharmacological analysis of the behavioral and biochemical effects of the $GABA_A$ receptor complex ligands could help to better characterize the mechanisms of the central effects of neurosteroids, their interactions with other neurotransmitter systems, and to better predict their potential for the treatment of neurological and psychiatric disorders, as well as side-effect profile.

2. Materials and methods

2.1. Animals

The experiments were carried out on adult male albino Swiss mice weighing 20–25 g. All animals were acclimatized to their cages for 5 days before testing. They were housed under a 12-h light–dark cycle, at a controlled temperature (20 °C), with water and food ad libitum. All experiments were done between 11:00 a.m. and 4:00 p.m. The experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609 EEC). All experimental procedures using animal subjects were approved by the Committee for Animal Care and Use at the Institute of Psychiatry and Neurology.

2.2. Convulsant test

Picrotoxin was dissolved in 0.9% NaCl and administered intraperitoneally (10 ml/kg). The mice were placed

singly in Plexiglas cages (20 × 25 × 15 cm) immediately after convulsant injection and observed for 30 min for the occurrence of the following signs: wild running and jumping, posturing (Straub tail), clonic convulsions (repetitive movements involving all limbs simultaneously). The seizures increased in severity and frequency and eventually progressed to status epilepticus, loss of righting response, tonic hindlimb extension and death. The pro-convulsive potency of a chemoconvulsant was defined as the percentage of animals showing consistent seizures leading to death within 30 min after the administration of picrotoxin. For subsequent experiments, the dose of picrotoxin was chosen to be within its LD_{85}–LD_{95} limits (effective lethal dose in 85–95% of mice), as determined during preliminary experiments.

2.3. Surgical procedure and microinjections

The i.c.v. injections of drugs were performed according to the procedure described previously (Herman, 1975; Turski and Stephens, 1992; Williams et al., 1995). Mice were anaesthetized with ketamine (50 mg/kg/10 ml, i.p.) and a sagittal incision was made along the midline of the skull. The bones were cleaned of connective tissue and the superior and transverse venous sinuses were identified. A small hole was made 2 mm caudal to the bregma and 2 mm lateral to the sagittal suture using a sharp needle. The hole was made by rotating the needle. The animals were tested further after a minimum of 4 days recovery. Microinjections were given unilaterally using a Hamilton microsyringe through a 3-mm long injection needle. All compounds were injected in a volume of 5 μl/50 s. The injection needle was removed after 30 s, and 10 min later, picrotoxin was administered intraperitoneally. The injection site was checked by injection of methylene blue solution (5 μl/50 s) according to the above procedure on the last day of the experiment, and 10 min prior to decapitation of the animals.

2.4. Drugs

The following drugs were used: midazolam maleate (Hoffman La Roche, Switzerland), 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) (RBI, USA), picrotoxin (Sigma-Aldrich, Poland). Midazolam was dissolved in distilled water. Allopregnanolone was suspended in 45% 2-hydroxypropyl-β-cyclodextrin (RBI) and was sonicated for 30 min before administration.

2.5. Administration regimen

The drugs were given either once or repeatedly at their ED_{85} (the dose inhibiting convulsions in 85% of animals), established in the acute part of the experiment. In the chronic study, the ED_{85} dose was administered once or twice daily to separate groups of animals, for 5 days. Picrotoxin was injected i.p. 10 min after the last injection of midazolam or allopregnanolone.

2.6. Biochemical analysis of monoamines and amino acids concentrations

The midazolam (38.25 nmol) and allopregnanolone (26.34 nmol) ED_{50} values determined in the acute dose–response part of the study were subsequently used for the biochemical analysis. The drugs and a solvent were administered i.c.v. 30 min before decapitation. The mouse brains were rapidly removed and the striatum was dissected bilaterally and frozen at −70 °C. Dopamine, 3,4-dihydroxyindolacetic acid (DOPAC), homovanilic acid (HVA),

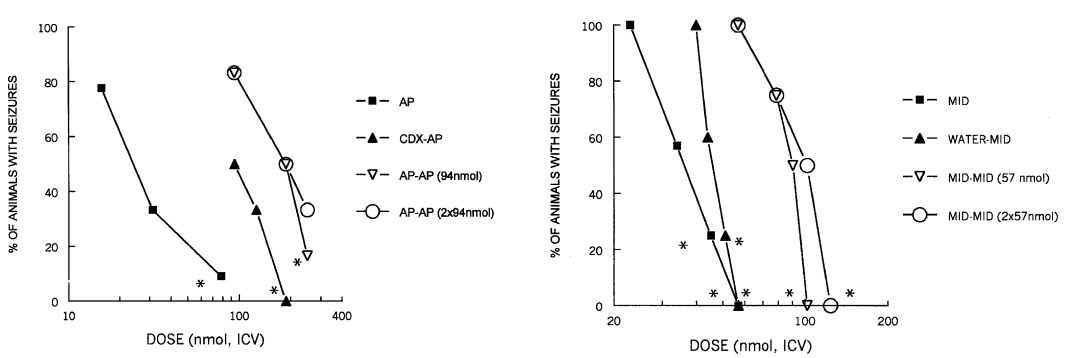

Fig. 1. Effects of acute and repeated i.c.v. injections of midazolam (MID) and allopregnanolone (AP) against seizures induced by i.p. injections of picrotoxin (12 mg/kg). The dose of neurotoxin was within the LD_{85}–LD_{95} limits. Each data point indicates the percentage of animals with seizures. MID and AP were injected i.c.v. 10 min before the convulsant administration. MID—animals treated acutely with midazolam; AP—animal treated acutely with allopregnanolone; WATER–MID—animals pretreated repeatedly with a solvent, and acutely with midazolam; CDX–AP—animals pretreated repeatedly with 45% 2-hydroxypropyl-β-cyclodextrin, and acutely with allopregnanolone; MID–MID (57 nmol)—animals pretreated repeatedly with midazolam once daily for 5 days at the ED_{85} dose, and acutely with the same drug on the last, 6th, day; AP–AP (94 nmol)—animals pretreated repeatedly with allopregnanolone once daily for 5 days at the ED_{85} dose, and acutely with the same drug on the last, 6th, day; MID–MID (2 × 57 nmol)—animals pretreated repeatedly with midazolam at the ED_{85} dose twice daily for 5 days, and acutely with the same drug on the last, 6th, day; AP–AP (2 × 94 nmol)—animals pretreated repeatedly with allopregnanolone at the ED_{85} dose twice daily for 5 days, and acutely with the same drug on the last, 6th, day. $n = 6$–8 mice per one data point. $^{*}P < 0.05$ compared to the convulsant itself.

Table 1
Effects of acute and repeated (5 days) injections (i.c.v.) of midazolam (the drug was administered repeatedly at the ED_{85} dose = 57 nmol) and allopregnanolone (the drug was administered repeatedly at the ED_{85} dose = 94 nmol) against seizures induced by i.p. injections of picrotoxin (12 mg/kg)

Drug	Acute drug injection	Acute drug injection (after repeated injections with a solvent)	Repeated drug injections (once daily)	Repeated drug injections (twice daily)
Midazolam	38.25 (30.58–43.41)	45.27 (41.04–50.33)	86.35 (75.89–98.28)	94.14 (76.32–116.11)
Allopregnanolone	26.34 (13.03–53.28)	98.18 (71.06–135.68)	164.56 (107.47–252.00)	186.70 (118.15–294.96)

The dose of neurotoxin was within the LD_{85}–LD_{95} limits. The data represent the ED_{50} values in nmol with 95% confidence limits. The pretreatment time before picrotoxin injection was 10 min for both compounds.

3-methoxytyramine, serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were assayed using a fully automated high-pressure liquid chromatography system with electrochemical detection and standard biochemical methods (Stefański et al., 1993).

2.7. Data analysis

The ED_{50} values with 95% confidence limits (CL) were determined using a computerized version of the Litchfield and Wilcoxon procedure. Fisher's exact probability test was used for specific comparisons between treatments. In the biochemical assay, the data are shown as means ± S.E.M., and were checked statistically by one-way analysis of variance followed by the Least Significant Difference (LSD) test.

3. Results

Picrotoxin produced seizures and the lethal effect with an LD_{50} = 9.92 mg/kg (95% confidence limits; CL = 9.05–10.87). Subsequently, the dose of 12 mg/kg (LD_{85}) was selected for further experiments. After acute injections, midazolam (ED_{50} = 38.25 nmol) and allopregnanolone (ED_{50} = 26.34 nmol) produced similar and dose-dependent protection against picrotoxin-induced seizures (Fig. 1, Table 1).

After repeated administration of a solvent (water), midazolam inhibited picrotoxin-induced convulsions to a similar extent (ED_{50} = 45.27 nmol). On the other hand, after repeated injection of midazolam (once or twice daily at the ED_{85} dose = 57 nmol), the potency of this drug to block neurotoxin-induced seizures was clearly diminished (ED_{50} = 86.35 and 94.14 nmol, respectively).

After repeated administration of a solvent (45% 2-hydroxypropyl-β-cyclodextrin), allopregnanolone inhibited picrotoxin convulsions less potently, indicating the influence of cyclodextrin on the effect of the neurosteroid (allopregnanolone; ED_{50} = 98.18 nmol). In spite of this, it appeared that after repeated injections of allopregnanolone (once or twice daily at the ED_{85} dose = 94 nmol), the potency of this drug to block picrotoxin convulsions was further diminished (ED_{50} = 164.56 and 186.7 nmol, respectively), indicating the development of tolerance.

Midazolam applied at the ED_{50} dose, established in the behavioural part of the study, significantly decreased the concentrations of 3-methoxytyramine $\{F(2,21) = 9.68,\ P < 0.01\}$, 3,4-dihydroxyindolacetic acid $\{F(2,21) = 8.61,\ P < 0.01\}$, and homovanilic acid $\{F(2,21) = 10.09,\ P < 0.01\}$ in the mouse striatum, whereas the levels of dopamine and serotonin remained unchanged (Table 2). Post-hoc test showed that midazolam given at the dose of 38.25 nmol significantly decreased the levels of 3-methoxytyramine ($P < 0.05$), 3,4-dihydroxyindolacetic acid ($P < 0.05$), and homovanilic acid ($P < 0.05$), as well as diminished the turnover rate of dopamine (f) ($P < 0.05$). Allopreg-

Table 2
Effects of midazolam and allopregnanolone on the concentration of dopamine, serotonin and their metabolites, and the turnover rate of dopamine (f), in the mouse striatum. The drugs were administered acutely at the ED_{50} doses, established in the behavioral part of the experiment. The data are shown as means ± S.E.M. (ng/g tissue).

Group	N	Dopamine (ng/g tissue)	DOPAC (ng/g tissue)	3-MT (ng/g tissue)	HVA (ng/g tissue)	f [(HVA)/(DA)] × 100	5-HT (ng/g tissue)	5-HIAA (ng/g tissue)
Control	8	8471 ± 602	1018 ± 79	603 ± 44	1450 ± 132	17 ± 0.70	440 ± 44	189 ± 15
Midazolam	8	7202 ± 351	700 ± 47[a]	371 ± 27[a]	1030 ± 45[a]	14 ± 0.56[a]	531 ± 27	166 ± 11
Allopregnanolone	8	7650 ± 300	747 ± 40[a]	363 ± 56[a]	1101 ± 36	14 ± 0.56[a]	506 ± 31	165 ± 10
ANOVA		$F(2,21) = 2.15$	$F(2,21) = 8.61$, $P < 0.01$	$F(2,21) = 9.68$, $P < 0.01$	$F(2,21) = 7.26$, $P < 0.01$	$F(2,21) = 10.09$, $P < 0.01$	$F(2,21) = 1.81$	$F(2,21) = 1.20$

[a] $P < 0.05$, difference from control (LSD test).

nanolone administered at the dose of 26.34 nmol decreased the concentration of 3-methoxytyramine ($P < 0.05$), 3,4-dihydroxyindolacetic acid ($P < 0.05$), and the turnover rate of dopamine ($P < 0.05$), but not the homovanilic acid concentration, in the mouse striatum (Table 2).

4. Discussion

We now showed for the first time that tolerance developed rapidly to the anticonvulsant activity of a neuroactive steroid when it was administered i.c.v., repeatedly, and over several days. The anticonvulsant potency of allopregnanolone was already diminished after five daily administration of the drug at the ED_{85} dose established in the acute part of the experiment. This effect appeared in spite of the fact that the vehicle used for repeated i.c.v. drug injections, cyclodextrin, also influenced the action of allopregnanolone. The effect of cyclodextrin was unexpected, as no other such reports on the pharmacological action of this solubilizer were found in the literature. Importantly, it was shown that allopregnanolone, given repeatedly as cyclodextrin complex, produced a much less potent anticonvulsant effect than did a single dose of this neurosteroid administered against the background of chronic cyclodextrin injections.

These data indicate that allopregnanolone-induced tolerance is a selective, neurosteroid-induced phenomenon. It is important to note that very similar effects were produced by repeated i.c.v. injections of midazolam (at the ED_{85} dose established in the acute part of the experiment), confirming that rapid and strong tolerance to the anticonvulsant activity occurs on chronic administration of benzodiazepines. After a survey of MEDLINE database resources, it appears that this is the first report on the development of adaptive processes to the anticonvulsant effect of allopregnanolone, a very potent positive allosteric modulator of $GABA_A$ receptors. Our results suggest that neuroactive steroids may have a tolerance liability similar to that of benzodiazepines, and therefore could be of limited value in chronic seizure therapy.

Though not directly studied, it seems that the diminished anticonvulsant potency of allopregnanolone and midazolam is a pharmacodynamic effect, not related to the pharmacokinetic factors. Benzodiazepines do not induce their own metabolism, and tolerance has been observed even with rapidly metabolized benzodiazepines (e.g. midazolam), indicating that maintained blood levels are not necessary for tolerance induction (Boisse et al., 1990; Perrault et al., 1992). Neurosteroids are also intensively metabolized and eliminated rapidly from the brain (Kokate et al., 1998; Reddy and Rogawski, 2000b; Visser et al., 2000).

It can, therefore, be concluded that the mechanism accounting for the diminished anticonvulsant potency of midazolam and allopregnanolone is of the pharmacodynamic type. The i.c.v. route of drug administration and the short latency of their central effects also contribute to such an outcome.

Several other in vitro and in vivo studies, in agreement with the present results, have shown a development of tolerance of the $GABA_A$ receptors to benzodiazepines and neuroactive steroids (Miller et al., 1988; Płaźnik, 1995; Yu and Ticku, 1995a,b; Marshall et al., 1997). For example, mice receiving lorazepam became tolerant to decreases in open-field activity and rotarod performance by day 7 of treatment (Marshall et al., 1997). Receptor binding studies indicated a reduction in the number of [^{3}H]flunitrazepam binding sites in the cortex after chronic benzodiazepine administration (Miller et al., 1988). Decreases were also observed in chloride uptake from cortical synaptosomes and in muscimol-induced chloride uptake (Miller et al., 1988; Płaźnik, 1995). Likewise, chronic dosing with the neuroactive steroid, minaxolone, at a dose which produced acute sedative effects similar to those of temazepam, also resulted in the development of tolerance after 7 days of treatment (Marshall et al., 1997). The magnitude of this effect was very similar to that of the effect observed with the benzodiazepine. Tolerance to the behavioral effects of benzodiazepines and neurosteroids coincided with decreases in the efficacy of γ-aminobutyric acid, neurosteroids and benzodiazepines to potentiate γ-aminobutyric acid-induced $^{36}Cl^-$ influx in intact cultured mammalian cortical neurons (Yu and Ticku, 1995a,b). Chronic exposure of neuronal cultures to pregnanolone resulted in uncoupling of the γ-aminobutyric acid, barbiturate, benzodiazepine and neurosteroid sites (a decrease in allosteric interaction among various binding sites in the γ-aminobutyric acid/benzodiazepine receptor complex), as revealed by receptor binding studies as well as down-regulation of γ-aminobutyric acid and *t*-butylbicyclophosphorothionate-binding sites (TBPS) (Friedman et al., 1993; Yu and Ticku, 1995b). All these experimental data indicate that benzodiazepines and neuroactive steroids positively modulating $GABA_A$ receptor functions have very similar pharmacological profiles, including adaptive changes in the $GABA_A$ receptor complex.

The reasons for the reported differences in the development of tolerance to the anticonvulsant effects of repeatedly administered neurosteroids are not known (see Introduction). The lack of anticonvulsant tolerance to the neuroactive steroids, pregnanolone and ganaxolone (Kokate et al., 1998; Reddy and Rogawski, 2000b), or the enhancement of the anticonvulsant potency of ganaxolone in the model of finasteride-induced reduction of the threshold for pentylenetetrazol seizures, under conditions of persistently high serum progesterone (pseudopregnancy) (Reddy and Rogawski, 2000a), are difficult to explain, given the present state of knowledge about adaptive changes that occur in the $GABA_A$ receptor complex (see above). Apparently, the lack of anticonvulsant tolerance to pregnanolone and

ganaxolone in mice and rats reported by others (Kokate et al., 1998; Reddy and Rogawski, 2000b) cannot be generalized to other neurosteroids.

Our results with i.c.v. injected allopregnanolone clearly demonstrated the development of anticonvulsant tolerance in the model of picrotoxin-induced seizures. The central route of drug administration limited the role of changes in the pharmacokinetic parameters (i.e. peripheral tissue disposition and metabolism) as the underlying factors. Likewise, the short latency and duration of the central effects of allopregnanolone reduced the possibility of a genomic action of this neurosteroid. Moreover, because it is likely that the higher the dose used, the more rapidly tolerance would develop, it is possible that the results of other studies in which no tolerance was discovered could be explained in terms of use of doses below the ED_{50} of drugs.

Some data show that novel neuroactive steroids with a wide separation between specific and non-specific central effects, and a lack of tolerance, are generally less potent than allopregnanolone as $GABA_A$ receptor modulators. For example, Co 2-6749 was less potent than allopregnanolone to inhibit [^{35}S]TBPS binding to native and recombinant human receptor combinations (Vanover et al., 2000). Moreover, whereas the effects of benzodiazepines at the $GABA_A$ receptor isoforms are clearly influenced by subunit composition, neuroactive steroids do not require a strict subunit composition for activity (Lambert et al., 1995). Thus, the lack of receptor selectivity and the lower efficacy at the $GABA_A$ receptors may explain the pharmacological profile of some neurosteroids, including a wider separation of their central effects, decreased tolerance and abuse potential.

Central injections of midazolam and allopregnanolone also decreased the concentration of dopamine metabolites, as well as diminished the dopamine turnover rate (homovanilic acid/dopamine ratio), in the mouse striatum. This finding indicates a selective reduction of dopamine metabolism in the basal ganglia. Both drugs administered i.c.v. at their ED_{50} doses, established in the picrotoxin model of seizures, were found to produce very similar biochemical effects. The present data confirmed and extended other reports on the inhibitory influence of positive allosteric modulators of the $GABA_A$ receptor complex, including benzodiazepines, on the spontaneous and stress-induced dopamine release in brain structures, including frontal cortex, nucleus accumbens and striatum (Finlay et al., 1992; Takada et al., 1993; Murai et al., 1994). Likewise, neurosteroids with $GABA_A$ receptor antagonistic properties increased K^+ evoked [^{3}H]dopamine release from rat nucleus accumbens slices, whereas neurosteroid positive modulators of γ-aminobutyric acid exerted an opposite effect (Jaworska-Feil et al., 1998). What was seen is that both potent positive allosteric modulators of $GABA_A$ receptors, allopregnanolone and midazolam, produced almost identical biochemical effects after their administration at the ED_{50} doses, obtained in the behavioral part of the experiment.

Recently, Motzo et al. (1996) found that i.c.v. injections of midazolam, but not of allopregnanolone, reduced basal dopamine content in the rat striatum. However, this experiment was done with much lower doses of midazolam (10.0 μg = 23.77 nmol), and allopregnanolone (15.0 μg = 47.09 nmol), and therefore, these results cannot be compared directly with the present data.

This part of the study features the similarity of the central effects, including an interaction with the dopaminergic system, of the benzodiazepine receptor full agonist and the most potent centrally neuroactive steroid. Thus, their common propensity to modulate central functions related to the brain dopaminergic innervation is clearly indicated.

Collectively, the results of this study, along with recently published data on the prolongation by i.c.v. injected allopregnanolone and midazolam of ethanol-induced sleep (Członkowska et al., 2000), indicate similar pharmacological and side-effects profiles of benzodiazepines and neurosteroids. Moreover, a similar efficacy of allopregnanolone and midazolam at the $GABA_A$ receptors has been found. These findings, together with the conversion of neurosteroids in the brain to other steroid hormones (testosterone, estradiol, and aldosterone) (Baulieu, 1998), add to the accumulating evidence suggesting a less favorable pharmacological profile for this class of drugs than was previously thought.

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