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Affinities of barbiturates for the GABA-receptor complex and A_1 adenosine receptors: a possible explanation of their excitatory effects

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Summary. The effects of barbiturates on the GABA-receptor complex and the A_1 adenosine receptor were studied. At the GABA-receptor complex the barbiturates inhibited the binding of [^{35}S]t-butylbicyclophosphorothionate ([^{35}S]TBPT) and enhanced the binding of [^{3}H]diazepam. Kinetic and saturation experiments showed that both effects were allosteric. Whereas all barbiturates caused complete inhibition of [^{35}S]TBPT binding, they showed varying degrees of maximal enhancement of [^{3}H]diazepam binding; (±)methohexital was identified as the most efficacious compound for this enhancement. At the A_1 adenosine receptor all barbiturates inhibited the binding of [^{3}H]N^6-phenylisopropyladenosine ([^{3}H]PIA) in a competitive manner. The comparison of the effects on [^{3}H]diazepam and [^{3}H]PIA binding showed that excitatory barbiturates interact preferentially with the A_1 adenosine receptor, and sedative/anaesthetic barbiturates with the GABA-receptor complex. It is speculated that the interaction with these two receptors might be the basis of the excitatory versus sedative/anaesthetic properties of barbiturates.

Key words: GABA-receptor complex – Adenosine receptors – Barbiturates

Introduction

Barbiturates are widely used as anaesthetic, sedative and anticonvulsant drugs. They have three distinct properties which can – to a varying extent – be found in any member of this group: these are the sedative/anaesthetic, the anticonvulsant and the excitatory effects (Nicoll 1978).

Several theories have been put forward to explain the mechanism of their CNS-depressant actions. These theories can be subdivided into those which favour a relatively nonspecific effect such as perturbation of membrane lipids (Seeman 1972), and those that assume a more specific interaction with membrane proteins, in particular with hormone or neurotransmitter receptors (LaBella 1981). A large body of evidence has been accumulated that the sedative/anaesthetic effects of barbiturates might be caused by such a specific interaction with the GABA-receptor-complex, which has been reviewed by Olsen (1982). This complex appears to contain a chloride channel as well as binding sites for GABA, benzodiazepines and convulsants like picrotoxinin (Olsen 1982). A good correlation between the effects of barbiturates on radioligand binding to the GABA receptor-complex, the enhancement of chloride efflux and the anaesthetic potency suggests that this is the site of the sedative and anaesthetic effects of barbiturates (Schwartz et al. 1985). The low efficacy of phenobarbital at this receptor, however, indicates that the GABA-receptor complex does not mediate the anticonvulsant effect of barbiturates (Schwartz et al. 1985; Leeb-Lundberg and Olsen 1982); this supports the early concept of distinct sedative/anaesthetic and anticonvulsant properties of barbiturates (see Harvey 1985).

In addition to these inhibitory effects on the central nervous system, barbiturates also possess excitatory properties. These can be seen with almost any barbiturate, but are more prominent with certain derivatives (Nicoll 1978). From a study on the effects of several inhibitory and excitatory barbiturates, Downes et al. (1970) concluded, that these two effects must be mediated by different mechanisms. Similar conclusions have been drawn from studies showing that several excitatory barbiturates interact with the GABA-receptor complex in a similar manner as do inhibitory barbiturates (Leeb-Lundberg and Olsen 1982). However, the mechanism responsible for the excitatory effects has so far not been identified.

Recently we have reported that barbiturates interact with A_1 adenosine receptors in the range of pharmacologically relevant concentrations (Lohse et al. 1984a). A_1 adenosine receptors mediate a variety of biological effects of adenosine. In the central nervous system they are responsible for the inhibitory effects of adenosine such as the reduction of neuronal firing, the inhibition of neurotransmitter release, and – in intact animals – sedative and anticonvulsant effects (Dunwiddie 1985). Barbiturates act as competitive antagonists at the A_1 receptor (Lohse et al. 1985). Therefore it is tempting to speculate that the excitatory effects of barbiturates are mediated by A_1 receptors, whereas the inhibitory effects are mediated via the GABA-receptor complex.

In order to test this hypothesis we undertook the present study to determine the affinities and efficacies of a series of barbiturates at the GABA-receptor complex and the A_1 receptor and compare these effects with their pharmacological properties.

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Abbreviations: GABA, γ-aminobutyric acid; TBPT, t-butylbicyclophosphorothionate; DMBB, 5-(1,3-dimethyl)butyl-5-ethylbarbituric acid; MCB, N-methyl-5-(1-cyclohexen-1-yl)-5-ethylbarbituric acid; MPPB, N-methyl-5-phenyl-5-propylbarbituric acid; PIA, N^6-phenylisopropyladenosine

Experimental procedures

Materials

[^{35}S]TBPT (90.2 Ci/mmol), unlabelled TBPT, [^{3}H]diazepam (70.0 Ci/mmol) and [^{3}H]PIA (49.9 Ci/mmol) were obtained from New England Nuclear, Dreieich, FRG. The stereoisomers of N-methyl-5-(1-cyclohexen-1-yl)-5-ethylbarbituric acid (MCB), N-methyl-5-phenyl-5-propylbarbituric acid (MPPB), hexobarbital and mephobarbital were kind gifts of Prof. Knabe, Saarbrücken, FRG, and the stereoisomers of pentobarbital were kindly provided by Prof. Carney, Oklahoma. All other materials were from sources previously described (Lohse et al. 1984a, 1985).

Methods

Preparation of rat brain membranes. Membranes from rat forebrains were prepared by a method adapted from Squires et al. (1983), using the centrifugation steps described earlier (Lohse et al. 1985). In brief, whole forebrains from male Wistar rats were placed in 0.32 M sucrose at 0° C; the tissue was homogenized with a glass/teflon homogenizer at 500 rpm for 30 s. The homogenate was centrifuged at 1,000 × g for 10 min and the resulting supernatant again centrifuged at 100,000 × g for 30 min. The pellet was resuspended in 1 mM EDTA/Tris, pH 7.4, and dialyzed four times against 50 vol. of double-distilled water at 4° C for 2 h each. After the dialysis the membranes were pelleted by centrifugation at 100,000 × g for 30 min, and the pellets were resuspended in the buffers used for the respective experiments (see below) at a protein concentration of 5 – 10 mg/ml. The protein concentration was determined by a modification of the Lowry method as described by Peterson (1977).

Radioligand binding assays. All radioligand binding experiments were done under conditions reported to be optimal for the individual binding assay. The radioligand concentration was always below the K_D-value of the radioligand.

The binding of [^{35}S]TBPT, a convulsant that binds to and acts via the picrotoxinin-binding site of the GABA-receptor complex, to rat brain membranes was measured with some modifications as described by Squires et al. (1983). The membranes (100 – 150 µg protein) were incubated with the radioligand in 250 µl of 50 mM Tris/HCl, pH 7.5, containing 200 mM KBr. Unless indicated otherwise the radioligand concentration was 1 nM. The higher concentration of buffer compared to the 5 mM used by Squires et al. (1983) was necessary to maintain a constant pH in the presence of barbiturates. For equilibrium binding experiments the incubation at 25°C lasted for 2 h and was terminated by filtration through Whatman GF/B glass-fibre filters. Nonspecific binding was defined by the presence of 100 µM picrotoxin and amounted to about 15% of the total binding at 1 nM [^{35}S]TBPT.

The binding of [^{3}H]diazepam to the benzodiazepine-binding site of the GABA-receptor complex was measured at 0°C as described by Leeb-Lundberg and Olsen (1982) using 50 mM sodium phosphate buffer, pH 7.0, containing 200 mM NaCl. The radioligand concentration was 0.5 nM.

The binding of the A_1 adenosine receptor agonist [^{3}H]PIA (1 nM) to rat brain membranes was measured at 25°C as described earlier (Lohse et al. 1985). 50 mM Tris/HCl, pH 7.4, was used as the incubation buffer. Endogenous adenosine was removed by the addition of 0.2 U/ml adenosine deaminase.

Adenylate cyclase assays. The activity of adenylate cyclase of rat brain membranes was determined as described (Lohse et al. 1985).

Data analysis. Equilibrium binding data were analyzed by non-linear curve-fitting with the program SCTFIT (De Lean et al. 1982) providing parameter estimates for binding to multiple sites. Kinetic binding data were fitted by non-linear regression to mono- or multiexponential equations (Lohse et al. 1984b).

Other concentration-response curves were analyzed by non-linear curve-fitting to the Hill-equation as described (Lohse et al. 1986). The EC_{50}-values of such curves denote the ligand concentration leading to the half-maximal effect of the individual curves. In the case of the enhancement of [^{3}H]diazepam binding, $EC_{15\%}$-values indicate the ligand concentration causing an enhancement by 15% of the basal value.

All data are from at least three independent experiments with duplicate samples, and are given as means ± SEMs or with 95% confidence limits.

Results

[^{35}S]TBPT bound in a saturable manner to dialyzed brain membranes, with a K_D-value of 33 nM (30 – 36 nM) and a binding capacity B_{max} of 690 ± 55 fmol/mg protein. The GABA-agonist muscimol inhibited the binding with an IC_{50}-value of 73 nM, whereas the GABA-antagonist bicuculline was without any effect in concentrations up to 10 µM; this indicates an intact GABA-ergic modulation of the binding of [^{35}S]TBPT and the essentially complete removal of endogenous GABA from the membrane preparation (Squires et al. 1983).

The specific binding of [^{35}S]TBPT was inhibited in a concentration-dependent manner by all barbiturates tested with the exception of barbituric acid itself. Figure 1 shows the inhibition curves obtained with the sedative/anaesthetic barbiturates (±)methohexital and (±)pentobarbital and the anticonvulsant phenobarbital. All three inhibited the binding of [^{35}S]TBPT with Hill coefficients of 1. The three barbiturates (as well as all other barbiturates tested) caused complete inhibition of [^{35}S]TBPT binding at higher concentrations, but the affinities were markedly different.

It has been suggested that the enhancement of [^{3}H]diazepam binding by barbiturates can be used to estimate not only their affinity, but also their efficacy at the GABA-receptor complex (Leeb-Lundberg and Olsen 1982). Figure 2 shows that the three barbiturates, which led to the same full inhibition of [^{35}S]TBPT binding, had very different efficacies in enhancing [^{3}H]diazepam binding. (±)Methohexital caused an enhancement by almost 150%, and pentobarbital by more than 100%, whereas phenobarbital had no effect. Such differences have led to the concept of full and partial agonist properties of barbiturates (Leeb-Lundberg and Olsen 1982). However, the order of potency of the three barbiturates was the same in the two experiments.

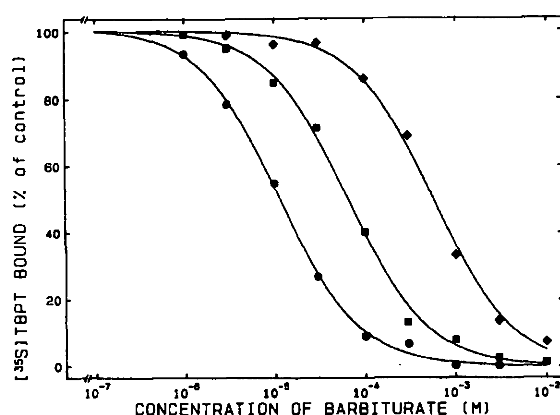

Fig. 1. Inhibition of [^{35}S]TBPT binding by barbiturates. The binding of [^{35}S]TBPT to rat brain membranes was measured as described under Methods in the presence of various concentrations of (±)methohexital (●), (±)pentobarbital (■), and phenobarbital (◆). The control value was 18.7 ± 1.4 fmol/mg protein. Data representing the means of 3 experiments were fitted to the Hill equation

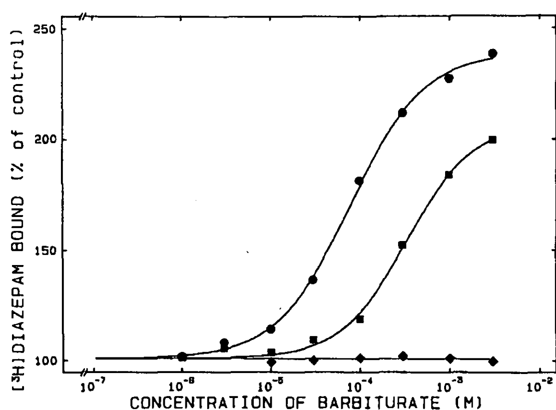

Fig. 2. Effects of barbiturates on the binding of [^{3}H]diazepam. The binding of [^{3}H]diazepam to rat brain membranes was measured as described under Methods in the presence of various concentrations of (±)methohexital (●), (±)pentobarbital (■), and phenobarbital (◆). The control value was 93.5 ± 4.7 fmol/mg protein. Data representing the means of 3 experiments were fitted to the Hill equation

In accordance with earlier reports (Leeb-Lundberg et al. 1980; Skolnick et al. 1981), we found that the enhancement of [^{3}H]diazepam binding by barbiturates was due to an increase in affinity without alterations in the binding capacity (data not shown). Both competitive and non-competitive mechanisms have been reported for the inhibition of [^{35}S]TBPT binding by barbiturates (Ramanjaneyulu and Ticku 1984; Trifiletti et al. 1985). We studied this question in kinetic and equilibrium saturation experiments.

The dissociation kinetics of [^{35}S]TBPT binding was the same whether initiated by a saturating concentration of unlabelled ligand or of picrotoxin (Fig. 3). However, the dissociation following the addition of a high concentration of a barbiturate was extremely rapid and almost complete within 15 s. This was observed with either the barbiturate alone or the barbiturate together with a high concentration of unlabelled TBPT or of picrotoxin. The dissociation

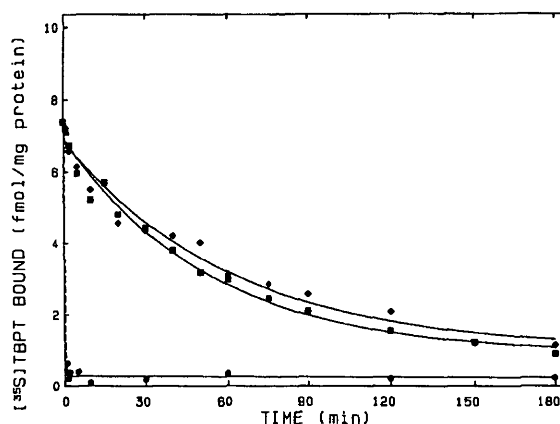

Fig. 3. Dissociation kinetics of [^{35}S]TBPT binding. After achievement of equilibrium of [^{35}S]TBPT binding to rat brain membranes, the dissociation of the radioligand was initiated by the addition of 1 µM unlabelled TBPT (◆), 100 µM picrotoxin (■), or 1 mM (±)methohexital (●). The data representing means of 3 experiments were fitted to a monoexponential equation giving the following dissociation constants: k_{-1} 0.017 min^{-1} (TBPT), k_{-1} 0.018 min^{-1} (picrotoxin). No fit could be obtained for methohexital ($k_{-1} > 10$ min^{-1}). The use of multiphasic equations did not lead to a significant improvement of the fits

Table 1. Effects of methohexital on equilibrium binding of [^{35}S]TBPT. The saturation of [^{35}S]TBPT binding (1 – 100 nM) to rat brain membranes was measured under control conditions and in the presence of 30 µM (±)methohexital. The data were analyzed by nonlinear curve-fitting. In both cases, only one component was detected, as also indicated by a linear Scatchard plot. Values represent means ± SEMs or 95% confidence intervals of 3 experiments

	B_{max} (fmol/mg protein)	K_D (nM)
Control	514 ± 51	29.8 (26.1 – 34.0)
(±)Methohexital (30 µM)	85 ± 22	26.2 (19.7 – 34.9)

kinetics suggest that the barbiturate does not act at the same site as TBPT or picrotoxin. Saturation experiments in the absence and presence of 30 µM methohexital confirmed the hypothesis of a non-competitive inhibition (Table 1): Methohexital caused a marked reduction of the binding capacity without affecting the affinity of the remaining binding sites.

Table 2 summarizes the affinity and efficacy of a series of barbiturates at the GABA-receptor complex as determined from the inhibition of [^{35}S]TBPT binding and the enhancement of [^{3}H]diazepam binding. On the whole, the order of potency determined by the two methods agrees relatively well, although the EC$_{50}$-values of the enhancement of [^{3}H]diazepam binding are generally higher. For several barbiturates there is a marked stereoselectivity. The efficacy of the barbiturates ranged from 0 to almost 150% enhancement of [^{3}H]diazepam binding.

The EC$_{50}$-values of the individual curves do not allow a comparison between the barbiturates, as they do not take into account the markedly different maximal effects. In such cases, the calculation of the ligand concentration causing a constant absolute enhancement can be used. We chose for

Table 2. Effects of barbiturates on [^{35}S]TBPT and [^{3}H]diazepam binding. E_{man} denotes the maximal enhancement above basal value in %. The basal value was 94 ± 5 fmol/mg protein. Values are means ± SEMs or 95% confidence intervals of 3 to 5 experiments with triplicate determinations

Barbiturate	[^{35}S]TBPT	[^{3}H]Diazepam	
	IC_{50} (μM)	EC_{50} (μM)	E_{max} (%)
(±)Methohexital	12 (8– 20)	69 (62– 78)	144 ± 4
(±)Thiopental	21 (12– 37)	111 (105– 118)	99 ± 7
(±)Pentobarbital	63 (54– 73)	302 (241– 379)	111 ± 8
(±)Secobarbital	81 (69– 97)	147 (120– 180)	104 ± 3
(±)Hexobarbital	130 (103– 170)	277 (155– 495)	41 ± 8
Amobarbital	140 (132– 152)	419 (269– 652)	91 ± 8
Phenobarbital	360 (275– 463)	–	0
Stereoisomers			
(–)DMBB	21 (14– 34)	35 (32– 39)	82 ± 6
(+)DMBB	55 (40– 75)	108 (67– 174)	49 ± 9
(–)Pentobarbital	60 (39– 91)	232 (179– 300)	121 ± 10
(+)Pentobarbital	95 (58– 156)	271 (184– 397)	71 ± 16
(–)Mephobarbital	35 (25– 50)	103 (87– 120)	46 ± 5
(+)Mephobarbital	465 (374– 578)	–	0
(–)MPPB	67 (30– 146)	195 (139– 273)	31 ± 6
(+)MPPB	720 (373–1,394)	–	0
(–)MCB	80 (40– 165)	493 (311– 782)	92 ± 8
(+)MCB	365 (193– 690)	705 (406–1,224)	43 ± 9
(+)Hexobarbital	110 (58– 194)	339 (231– 497)	41 ± 10
(–)Hexobarbital	340 (228– 507)	1,434 (1,297–1,585)	47 ± 13

this purpose an enhancement by 15%, representing the half-maximal effect of (–)MPPB, which is of the sedative/anaesthetic barbiturates the one causing the least enhancement of [^{3}H]diazepam binding. These $EC_{15\%}$-values are used for the comparison with the effects of barbiturates at A_1 adenosine receptors.

We have previously shown that barbiturates are competitive antagonists at A_1 adenosine receptors (Lohse et al. 1985). None of the barbiturates used in this study had any agonist activity at A_1 receptors, as evidenced by a lack of adenylate cyclase inhibition in both rat brain and fat cell membranes (data not shown). The affinity of the barbiturates for the A_1 receptor was tested in competition experiments for the binding of [^{3}H]PIA to rat brain membranes. Because of their full antagonist properties, their affinities determined in the competition experiments were used for a comparison with their effects at the GABA-receptor complex.

The effects of the barbiturates on the A_1 receptor and the GABA-receptor complex are compared in Table 3. The selectivity for the GABA-receptor complex was calculated by dividing the K_i-values by the respective $EC_{15\%}$-values. Table 3 also gives data from the literature on the pharmacological properties (sedative/anaesthetic vs. excitatory) of the individual compounds, as observed in studies with intact animals (see references in Table 3).

The top group lists barbiturates which are used in clinical practice as sedatives/anaesthetics. All these compounds have a preferential action at the GABA-receptor complex, as indicated by a "GABA-selectivity" factor above 1. In contrast, phenobarbital, which is used because of its anticonvulsant properties, does not cause a measurable enhancement [^{3}H]diazepam binding.

The second group comprises the stereoisomers of pentobarbital and hexobarbital, which all have sedative/anaesthetic properties. In both pairs the more potent anaesthetic isomer (Wahlström 1966; Büch et al. 1969) had both a lower $EC_{15\%}$ in enhancing [^{3}H]diazepam binding and a somewhat higher selectivity for the GABA-receptor complex.

Thirdly, we investigated a number of stereoisomers, which produce opposite pharmacological effects. In each of these pairs, the sedative/anaesthetic isomer had a preferential action at the GABA-receptor complex, whereas the excitatory isomer had a higher affinity for the A_1 receptor. Two of these excitatory isomers had no measurable effect on the binding of [^{3}H]diazepam, although at high concentrations they inhibited [^{35}S]TBPT binding.

Discussion

The understanding of the molecular mechanisms of barbiturate effects is still very incomplete. A lot of pharmacological evidence suggests that the three major effects – sedative/anaesthetic, anticonvulsant and excitatory – are mediated by different mechanisms (Leeb-Lundberg and Olsen 1982; Harvey 1985; Downes et al. 1970). Although several authors assume that relatively non-specific interactions of barbiturates with the plasma membrane form the basis of the depressant effects (Seeman 1972), this theory cannot account for the marked differences of several pairs of stereoisomers. In fact, these differences suggest more specific interactions in particular with membrane-bound receptors (Andrews and Mark 1982).

Table 3. Affinities of barbiturates for A_1 adenosine receptors and GABA-receptor complexes

Barbiturate	A_1 receptor [^{3}H]PIA K_i (μM)	GABA receptor [^{3}H]diazepam $EC_{15\%}$ (μM)	GABA-selectivity K_i (A_1)/ $EC_{15\%}$ (GABA)	Pharmacological effect[a] S; E; AC
(±)Methohexital	340	9	38	S
(±)Thiopental	170	41	4.2	S
(±)Secobarbital	120	42	2.9	S
(±)Hexobarbital	620	210	2.9	S
(±)Pentobarbital	110	70	1.6	S
Amobarbital	150	110	1.4	S
Phenobarbital	360	∞[b]	0	AC
Stereoisomers with similar pharmacology				
(−)Pentobarbital	91	37	2.5	S
(+)Pentobarbital	230	115	2.0	S
(+)Hexobarbital	520	250	2.1	S
(−)Hexobarbital	720	500	1.5	S
Stereoisomers with different pharmacology				
(−)MCB	1 070	105	10	S
(+)MCB	310	410	0.7	E
(−)Mephobarbital	580	67	8.4	S
(+)Mephobarbital	350	∞	0	E
(−)DMBB	26	13	2.0	S
(+)DMBB	60	80	0.7	E
(−)MPPB	260	200	1.3	S
(+)MPPB	520	∞	0	E

[a] The barbiturates are classified as either sedative/anaesthetic (S), excitatory (E), or anticonvulsive (AC) according to the following reports: Harvey (1985); Downes et al. (1970); Wahlström (1966); Wahlström and Norberg (1984): Büch et al. (1968, 1969, 1970, 1973)
[b] ∞ indicates that the compound does not enhance [^{3}H]diazepam binding

We have investigated the effects of barbiturates on two such receptors, the GABA-receptor complex and the A_1 adenosine receptor. At the GABA-receptor complex, barbiturates inhibited the binding of [^{35}S]TBPT, as reported by others (Squires et al. 1983; Ramanjaneyulu and Ticku 1984; Trifiletti et al. 1985). In contrast to the reports by Ramanjaneyulu and Ticku (1984) and Trifiletti et al. (1985) we observed no effect of methohexital on the K_D-value of [^{35}S]TBPT, resulting in a truly non-competitive inhibition. Together with the markedly different dissociation kinetics following methohexital on the one side, and picrotoxin or unlabelled TBPT on the other side, this suggests that barbiturates act via a site distinct from the TBPT-binding site. The inhibition of [^{35}S]TBPT binding seems to occur via an allosteric mechanism.

The enhancement of [^{3}H]diazepam binding by barbiturates occurred also via an allosteric mechanism (Leeb-Lundberg and Olsen 1982; Skolnick et al. 1981). However, although all barbiturates were equally effective in inhibiting the binding of [^{35}S]TBPT, they had very different efficacies in enhancing the binding of [^{3}H]diazepam. The highest efficacy had methohexital, which caused significantly greater enhancement than the previously identified most effective compounds pentobarbital and secobarbital (Leeb-Lundberg and Olsen 1982). Therefore, with respect to methohexital all other barbiturates must be regarded as partial agonists with varying efficacies. Some of them, for example phenobarbital, produced no measurable enhancement of [^{3}H]diazepam binding at all. However, this may be due to the fact that high concentrations (> 10 mM) of most barbiturates inhibit the binding of [^{3}H]diazepam, which is most likely due to an alkalinisation of the incubation buffer by high concentrations of barbiturates. Such effects may obscure a minor enhancement of [^{3}H]diazepam binding, so that we cannot exclude a very low intrinsic activity of those barbiturates which produced no measurable enhancement.

It has been suggested that the enhancement of [^{3}H]diazepam binding is related to the sedative/anaesthetic effects of barbiturates (Leeb-Lundberg and Olsen 1982), which is supported by a correlation of this effect both with an enhancement of chloride-fluxes and anesthetic potency (Schwartz et al. 1985; Leeb-Lundberg et al. 1980). Whereas those barbiturates which produced no measurable enhancement of [^{3}H]diazepam binding have only little or no sedative properties, some of the known sedative/anaesthetic barbiturates, for example (±)hexobarbital, produce only a small maximal enhancement of [^{3}H]diazepam binding. Therefore we chose the $EC_{15\%}$-values as the basis for the comparison of different barbiturates as detailed in the results section. This would suggest that a minor enhancement of [^{3}H]diazepam binding corresponds already to anaesthesia. This view is supported by the fact that the $EC_{15\%}$-values of several barbiturates agree well with the free plasma concentrations found during anaesthesia; for example, during anaesthesia in the rat the free plasma concentration of (±)pentobarbital ($EC_{15\%}$ 70 μM) has been estimated at 80 μM (Lohse et al. 1985; Büch et al. 1969), of (−)MCB ($EC_{15\%}$ 105 μM) at 80−180 μM (Büch et al. 1970), and of (−)mephobarbital ($EC_{15\%}$ 67 μM) at 60−200 μM (Büch et al. 1968). Likewise, during anaesthesia in man, plasma levels of methohexital ($EC_{15\%}$ 9 μM) are in the range of 10 μM (Breimer 1976), and of thiopental ($EC_{15\%}$ 46 μM) in the range of 16−65 μM (Burch and Stanski 1983). Moreover, the $EC_{15\%}$-values of the enhancement of [^{3}H]diazepam binding also agree relatively well with the IC_{50}-values of the inhibition of [^{35}S]TBPT binding.

Whereas at the GABA-receptor complex only allosteric effects of barbiturates have been demonstrated, we have previously shown that at the A_1 adenosine receptor barbiturates interact with the binding site in a competitive manner (Lohse et al. 1985). In agreement with earlier results, all barbiturates appeared to be antagonists at this receptor. This is a property which they share with theophylline and caffeine, which are known for their stimulatory effects on the central nervous system. Therefore it was tempting to speculate that blockade of A_1 adenosine receptors by barbiturates might be the basis of their excitatory actions. Recently we have shown that barbiturates can enhance the release of neurotransmitters in the hippocampus by blocking presynaptic A_1 receptors (Lohse et al. 1987). This could be related to their excitatory actions. The resulting speculation would be that excitatory barbiturates preferentially block the A_1 receptor, whereas sedative/anaesthetic barbiturates preferentially act at the GABA-receptor complex.

The investigation of the effects of barbiturates at either receptor alone has not led to a possible explanation of their different pharmacological actions. Thus, it was noted that several excitatory barbiturates also had agonist properties

at the GABA-receptor complex as seen in an enhancement of [^{3}H]diazepam binding (Leeb-Lundberg and Olsen 1982). We observed such an enhancement for (+)DMBB and (+)MCB, but not for other excitatory isomers. This suggests that these compounds possess sedative/anaesthetic properties. On the other hand, whereas in several pairs of stereoisomers the excitatory isomer was more potent at the A_1 receptor than its sedative/anaesthetic counterpart, this was not the case with all of these pairs (Lohse et al. 1985).

However, when the effects on both receptor systems are taken together, we obtain data which are compatible with this hypothesis. In particular, the investigation of stereoisomers with opposite pharmacological effects lends support to this idea. In each of these pairs, the excitatory property correlated with a preferential action at the A_1 receptor, as indicated by "GABA-selectivity" factors below 1. In contrast, sedative/anaesthetic barbiturates had "GABA-selectivity" factors above 1. A particularly high selectivity for the GABA-receptor complex was calculated for methohexital, followed by thiopental, which agrees well with their usefulness in the induction of anaesthesia.

The anticonvulsant barbiturate phenobarbital had practically no activity at the GABA-receptor complex neither in our experiments nor in several other studies (Leeb-Lundberg et al. 1980; Schwartz et al. 1985; see also Harvey 1985). This agrees with the view mentioned in the introduction that the anticonvulsant effects of barbiturates are not mediated via the GABA-receptor complex. Possible reasons why minor effects of phenobarbital on [^{3}H]diazepam binding – which would correspond to its minor sedative effects – were not observed under our experimental conditions have been discussed above. However, it is interesting to note that the so-called paradoxical excitement is most frequently seen with phenobarbital (Harvey 1985). This might be related to the observation that – although therapeutic levels of phenobarbital (40–100 μM, Harvey 1985) are below its K_i-value at the A_1 receptor – phenobarbital has a GABA-selectivity factor of 0.

Obviously, our data do not exclude the possibility that other factors contribute to either the excitatory or the sedative/anaesthetic effects of barbiturates. In addition, they provide no clues on the mechanism of anticonvulsant action, as clearly seen with phenobarbital. However, they may provide a useful speculation on the mechanisms of the excitatory and sedative/anaesthetic actions. They also show that the simultaneous investigation of different biochemical mechanisms may be necessary to understand a pharmacological effect.

It remains to be seen, how the inputs from the GABA-receptor complex and the A_1 adenosine receptor could be integrated to produce an overall effect, which may be either excitatory or sedative/anaesthetic.

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