J. Pharm. Pharmacol. 1985, 37: 928–929
Communicated July 19, 1985

© 1985 J. Pharm. Pharmacol.

Phenobarbitone enhances [^{35}S]TBPS binding to extensively washed rat cortical membranes

TAGE HONORÉ*, JØRGEN DREJER, *A/S Ferrosan Research Division, Sydmarken 5, DK-2860 Soeborg, Denmark*

Pentobarbitone and phenobarbitone enhance [^{35}S]TBPS binding to extensively washed rat cortical membranes with ED50 values of 15 and 120 μM, respectively, indicative of an interaction with the chloride ionophore part of the GABA-receptor different from the TBPS binding site, but allosterically coupled to this site.

It has recently been demonstrated that benzodiazepine (BZ) receptor ligands up- and down-regulate the binding of [^{35}S]TBPS ([^{35}S]t-butylbicyclophosphorothionate) to extensively washed rat cortical membranes according to their pharmacological efficacies (Supavilai & Karobath 1983; Nielsen et al 1985). The barbiturates have many pharmacological properties similar to the benzodiazepines, e.g. anticonvulsant, anxiolytic and sedative effects, and it is believed that barbiturates exert their action via the BZ-/GABA-receptor-chloride ionophore complex probably by interaction with the ionophore part of the complex (Olsen 1982). Several reports have shown that barbiturates inhibit the binding of [^{35}S]TBPS to rat cortex (Squires et al 1983; Ramanjaneyulu & Ticku 1984), and that the inhibition by pentobarbitone is non-competitive and probably allosteric (Maksay & Ticku 1985; Richter & Yamamura 1985). In these experiments, however, the above mentioned extensively washed membrane preparation was not used.

The present study shows that pentobarbitone and phenobarbitone at low concentrations allosterically enhance binding of [^{35}S]TBPS to extensively washed rat cortical membranes. Higher concentrations of the barbiturates inhibit [^{35}S]TBPS binding as found by others (Squires et al 1983; Ramanjaneyulu & Ticku 1984).

Methods

Cerebral cortex (frozen overnight at −70 °C) from male Wistar rats (200–300 g) was thawed and homogenized in Tris citrate (50 mM; pH ~ 7·1) by an Ultra-Turrax homogenizer and centrifuged for 10 min at 27 000*g*. The pellet was washed by homogenization in the same buffer and centrifuged for 10 min at 27 000*g*. The final pellet was frozen overnight. This procedure of thawing, washing twice, and freezing overnight was repeated twice more. The third time, the final pellet was homogenized in Tris citrate (50 mM), sodium chloride (1 M) pH 7·1 (100 vol per g of original tissue weight) and used for the binding assay. Aliquots of 0·5 ml of tissue suspension containing 1 nM [^{35}S]TBPS (spec. act. 45·8 Ci mmol^{-1}, NEN) and drugs to be tested were incubated for 60 min at 25 °C. Separation of bound ligand was done after the addition of 10 ml of ice-cold buffer by rapid filtration through Whatman GF/C glass fibre filters under suction followed by washing with 10 ml ice-cold buffer. Radioactivity on filters was determined by liquid scintillation counting. Non-specific binding was defined as binding in the presence of picrotoxinin (30 μM) and was less than 15% of total binding.

DMCM (methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate) and ZK 93423 (ethyl 6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate) were synthesized by Schering AG, Berlin. Ro 15-1788 (ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo [1,5-*a*]-[1,4]benzodiazepine-3-carboxylate) and flurazepam were gifts from Roche, Basel. All other drugs and chemicals were from regular commercial sources.

Results and discussion

Pentobarbitone and phenobarbitone enhanced [^{35}S]TBPS binding dose dependently (Fig. 1) with EC50 = 15 and 120 μM, respectively. However, the maximal enhancement obtained with pentobarbitone was only 1·2-fold at 100 μM (data not shown) compared with phenobarbitone which gave a 1·7-fold enhancement of [^{35}S]TBPS binding at 500 μM (Table 1). The results

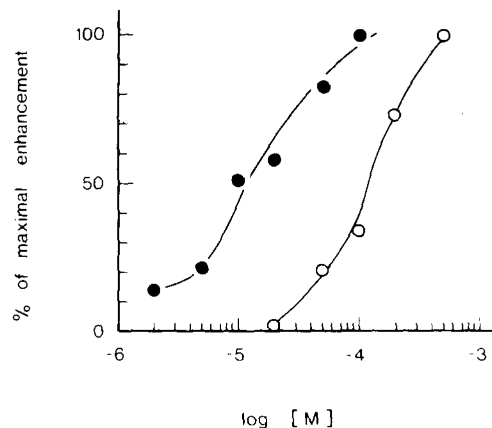

FIG. 1. Enhancement of [^{35}S]TBPS binding (final concentrations 1 nM) to extensively washed rat cortical membranes by pentobarbitone (●) and phenobarbitone (○). Maximal enhancement was 1·2-fold for pentobarbitone and 1·7-fold for phenobarbitone. The experiment was reproduced twice.

* Correspondence.

Table 1. Modulation of [^{35}S]TBPS (final concentration 1 nM) binding to extensively washed rat cortical membranes by BZ-receptor ligands phenobarbitone and combination of compounds.

	Specific [^{35}S]TBPS binding counts min^{-1}/assay ± s.e.m. (N)	% of control
Control	1099 ± 17 (19)	100
ZK 93423 100 nM	1783 ± 32 (8)	162
Flurazepam 100 nM	1390 ± 42 (8)	126
Ro 15-1788 10 μM	943 ± 19 (8)	86
DMCM 100 nM	641 ± 18 (4)	58
Ro 15-1788 10 μM		
+ ZK 93423 100 nM	887 ± 28 (4)	81
+ Flurazepam 100 nM	833 ± 15 (4)	80
+ DMCM 100 nM	863 ± 18 (4)	79
Phenobarbitone 500 μM	1895 ± 14 (8)	172
Phenobarbitone 500μM		
+ ZK 93423 100 nM	2065 ± 14 (8)	188
+ Flurazepam 100 nM	1996 ± 12 (8)	182
+ Ro 15-1788 10 μM	1240 ± 17 (8)	113

The experiment was reproduced four times.

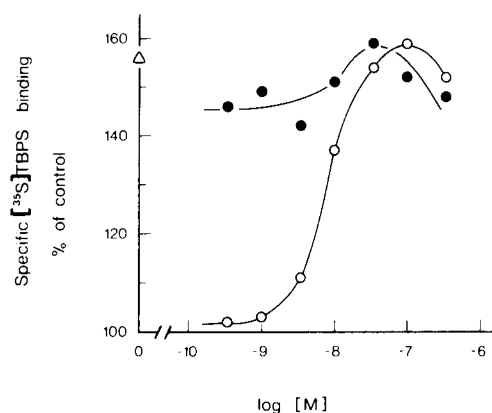

Fig. 2. The effect of a BZ-receptor agonist on [^{35}S]TBPS binding (final concentration 1 nM) to extensively washed rat cortical membranes. ZK 93423 alone (○), ZK 93423 in the presence of 500 μM phenobarbitone (●), 500 μM phenobarbitone alone (△). The experiment was reproduced twice.

suggest that barbiturates interact with the chloride ionophore via a binding site different from the TBPS binding site, but allosterically coupled to this binding site. Concentrations of the barbiturates ten times higher than used for maximal enhancement of [^{35}S]TBPS binding gave more than 90% inhibition of the binding (data not shown). The inhibition of [^{35}S]TBPS binding by very high concentrations of the barbiturates, which has also been found by others (Squires et al 1983; Ramanjaneyulu & Ticku 1984) might not be related to the pharmacological effect of these drugs.

Results shown in Table 1 confirm that BZ receptor ligands up- and down-regulate [^{35}S]TBPS binding. Thus the BZ-receptor agonists, ZK 93423 and flurazepam, enhance [^{35}S]TBPS binding 1·6- and 1·3-fold, respectively. The BZ-receptor inverse agonist, DMCM reduces [^{35}S]TBPS binding 1·7-fold. The up- and down-regulation of [^{35}S]TBPS binding was antagonized by the BZ-receptor antagonist Ro 15-1788.

ZK 93423 enhanced [^{35}S]TBPS binding dose dependently (Fig. 2). ZK 93423 in the presence of phenobarbitone 500 μM showed an enhancement similar to that obtained with phenobarbitone (500 μM) alone, independently of the dose of ZK 93423. These results suggest that allosteric regulation of the TBPS binding site has an upper limit irrespective of how many parts of the GABA-BZ-receptor chloride ionophore complex are activated. Alternatively, the results might indicate a common coupling mechanism of BZ-receptors and barbiturate binding sites and the TBPS binding site.

The BZ-receptor agonist ZK 93423 (100 nM) and flurazepam (100 nM) only enhanced TBPS binding marginally in the presence of phenobarbitone (500 μM) (Table 1). On the other hand, the BZ-receptor antagonist Ro 15-1788 (10 μM) reduced TBPS binding in the presence of phenobarbitone (500 μM). These results suggest that Ro 15-1788 interacts with the barbiturate site. Alternatively, Ro 15-1788 may have an effect on the coupling mechanism between the barbiturate binding site and the TBPS binding site.

In summary, the present study shows an allosteric effect of barbiturates on the TBPS binding site, which represents the chloride part of the GABA/BZ-receptor-chloride ionophore complex. Furthermore, this study demonstrates for the first time a marked neurochemical effect of phenobarbitone under in-vitro conditions.

The excellent technical assistance by Pia Birn, Ulla G. Olsen and Vibeke Petersen is gratefully acknowledged.

REFERENCES

Maksay, G., Ticku, M. K. (1985) J. Neurochem. 44: 480–486

Nielsen, M., Honoré, T., Braestrup, C. (1985) Biochem. Pharmacol. 34: 3633–3642

Olsen, R. W. (1982) Ann. Rev. Pharmacol. Toxicol. 22: 245–254

Ramanjaneyulu, R., Ticku, M. K. (1984) J. Neurochem. 42: 221–229

Richter, J. A., Yamamura, H. I. (1985) J. Pharmacol. Exp. Ther. 233: 125–133

Squires, R. F., Casida, J. E., Richardson, M., Saederup, E. (1983) Mol. Pharmacol. 23: 326–336

Supavilai, P., Karobath, M. (1983) Eur. J. Pharmacol. 91: 145–146