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Variations in the kinetic pattern of astrocytic γ-aminobutyric acid uptake when inhibited by different barbiturates

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γ-Aminobutyric acid (GABA) is removed from the extracellular space of the central nervous system by uptake into both neurons and astrocytes [1–4]. We have previously studied the astrocytic uptake in detail and found that it is a net uptake, not a homoexchange [5]. We have also shown that pentobarbital and certain other barbiturates inhibit both neuronal and astrocytic GABA uptake. The effect of pentobarbital is much more potent on astrocytes than on neurons, but it is not known whether clinically efficient concentrations are sufficient to inhibit the uptake [6, 7]. A similar inhibition by pentobarbital of GABA uptake into brain slices is known to be competitive [8], but no information is available about the kinetics of the inhibition of GABA uptake into astrocytes. Since drug-induced inhibition of GABA uptake into astrocytes seems to be of pharmacological interest [9–12], such information is of relevance. In the present work, therefore, kinetics of the inhibition of GABA uptake by different barbiturates were studied in astrocytes in primary cultures, which constitute a good model for their *in vivo* counterparts [13].

Methods

Cultures of astrocytes were prepared as previously outlined [1, 5, 11] and described in detail by Hertz *et al.* [13]: the parts of the cerebral hemispheres above the lateral ventricles were dissected and removed from the brains of newborn Swiss mice and grown for 3 weeks in tissue culture medium [modified Eagle's Minimum Essential Medium (MEM)] [1. 13] with serum and, during the last week, also with 0.25 mM dibutyryl cyclic AMP, a compound known to evoke a pronounced morphological differentiation of the cells [13].

Before uptake experiments the layer of astrocytes was carefully loosened with a soft Teflon spatula, dissected into samples corresponding to approximately 50 μg protein, and preincubated in 450 μl of a serum-free, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES)-buffered MEM containing the desired concentration of unlabeled GABA and of either pentobarbital, phenobarbital or aprobarbital. After 30 min of preincubation (37°), radioactive GABA ([2, 3-^{3}H]GABA; 35 Ci/mmole) was added, and the uptake of [^{3}H]GABA during 5 min was measured at 37°. Previous experiments have shown that the GABA uptake is linear during this length of time [1] and that the contribution to the uptake by homoexchange is negligible [5]. The incubation was terminated by centrifugation (10 sec) and rapid washing of the pellets (10 sec) with non-radioactive medium. The cells were dissolved in 100 μl of 2 N KOH, and after appropriate dilution the radioactivity was determined as previously described using a Packard TriCarb scintillation spectrometer [1].

Results and discussion

The effects of pentobarbital, phenobarbital or aprobarbital on GABA uptake at an external GABA concentration of either 1 or 20 μM are shown in Table 1. In accordance with previous results [6, 7], pentobarbital exerted a considerably larger inhibition than phenobarbital. Thus, pentobarbital inhibited GABA uptake even at the pharmacologically relevant concentration of 0.1 mM whereas phenobarbital at most evoked a marginal inhibition unless a very high concentration (3.0 mM) was used. Aprobarbital, which was found previously to possess little, if any, inhibitory effect without preincubation [7], did inhibit the uptake considerably even at relatively low doses in the present study in which the astrocytes had been preincubated with the drug for 30 min before the uptake experiment. In contrast to pentobarbital which, at least at a high concentration, had a larger effect at a GABA concentration of 1 μM than at a GABA concentration of 20 μM, aprobarbital caused a larger inhibition at the higher GABA concentration. This suggests differences in inhibition patterns, and a more detailed kinetic study of the effects of each of these two barbiturates, therefore, seemed warranted.

A kinetic study of inhibition patterns requires relatively distinct inhibition and, therefore, was carried out using only the highest barbiturate concentration (3.0 mM). The results of this study are given in Table 2. It is seen that the inhibitory patterns of the two barbiturates are distinctly different. Pentobarbital, at 3.0 mM, affected primarily the K_m value for GABA uptake suggesting competitive inhibition, whereas aprobarbital affected only V_{max} suggesting that it acts in a non-competitive manner. Using the apparent K_m values given in Table 2 and assuming that the inhibition exerted by pentobarbital was purely competitive, a K_i value of 0.8 mM was calculated. This K_i value is similar to that observed by Cutler *et al.* [8] in brain slices. However, the inhibition exerted by 0.1 mM pentobarbital at a GABA concentration of 20 μM (28.2 ± 7.5%) was larger than that which was calculated on the assumption of competitive inhibition with a K_i of 0.8 mM and the observed kinetic constants for GABA uptake (cf. Table 2). This supports our previous conclusion that "part of, but probably not the entire pentobarbital action is due to a competitive inhibition" [6]. In the case of aprobarbital, the actual inhibitory action may also be more complex than a purely non-competitive inhibition since at 3.0 mM the inhibition (Table 1) was more pronounced than could be anticipated from the data in Table 2 assuming strict linear, non-competitive inhibition. The same may be true in the case of phenobarbital (Table 1) although no detailed kinetic analysis was performed due to the relatively low potency of this barbiturate.

The inhibition of GABA uptake at 0.1 mM pentobarbital seems of special importance because this is the level of pharmacological relevance [15–18]. It is therefore likely that at least some of the pharmacological actions of barbiturates on the central nervous system *in vivo* might partly be due to an inhibition of GABA uptake into astrocytes [7] which, in turn, might enhance the action of endogenously released GABA [10, 11]. To what extent inhibition of the neuronal GABA uptake, which quantitatively is at least as important as that into astrocytes [2], is also involved is unknown. However, pentobarbital is a much more potent inhibitor of GABA uptake into cultured astrocytes than into cultured neurons where > 1 mM pentobarbital is required to inhibit the uptake [7]. Also, the inhibitory effect of pentobarbital on synaptosomal GABA uptake is negligible [19]. These observations do suggest that barbiturates affect astrocytic rather than neuronal GABA uptake. In any case, it seems the barbiturates exert their actions on brain function not only by interacting with the GABA receptor [20] which is exclusively localized on neurons [21, 22] but also with other GABA recognition sites

Table 1. Effects of pentobarbital, phenobarbital and aprobarbital on GABA uptake at 1 or 20 μM external GABA*

Concn of barbiturates (mM)	GABA uptake (% of control)					
	Pentobarbital		Phenobarbital		Aprobarbital	
	External GABA concn		External GABA concn		External GABA concn	
	1 μM	20 μM	1 μM	20 μM	1 μM	20 μM
0	100.0 ± 3.4† (8)	100.0 ± 4.6‡ (10)	100.0 ± 3.4† (8)	100.0 ± 4.6‡ (10)	100.0 ± 3.4† (8)	100.0 ± 4.6‡ (10)
0.10	89.1 ± 8.0 (5)	71.8 ± 7.5§ (5)			94.3 ± 7.4 (5)	92.9 ± 6.7 (4)
0.25	88.1 ± 4.3 (4)	83.1 ± 4.2‖ (4)	85.4 ± 3.1§ (5)	102.1 ± 7.5 (5)	101.9 ± 17.0 (4)	70.1 ± 9.3‖ (5)
1.0	87.7 ± 5.4 (5)	74.7 ± 1.9¶ (5)	105.6 ± 1.0 (4)	102.1 ± 17.2 (4)	74.0 ± 4.9§ (5)	69.7 ± 8.2§ (5)
3.0	57.4 ± 4.1¶ (5)	79.6 ± 4.2§ (4)	83.3 ± 5.3‖ (5)	56.4 ± 7.1¶ (4)	62.3 ± 5.7¶ (5)	38.2 ± 3.6¶ (5)

* Astrocytes were preincubated for 30 min (37°) in the presence of barbiturates and non-radioactive GABA. At the end of the preincubation period, radioactive GABA was added, and the incubation was continued for an additional 5 min. Uptake of radioactive GABA during this period was calculated, corrected for the non-saturable component [1], and expressed as a percentage of the control. Results are averages ± S.E.M. with numbers of experiments in parentheses.

† Absolute value 0.008 nmole/min per mg protein.

‡ Absolute value 0.124 nmole/min per mg protein.

§ $P < 0.025$.

‖ $P < 0.05$.

¶ $P < 0.001$.

Table 2. Kinetic constants of GABA uptake into cultured astrocytes in the absence or presence of either pentobarbital or aprobarbital*

Experimental condition	Apparent K_m (μM)	V_{max} (% of control)
Control	66.9 ± 10.0	100.0 ± 5.6
Pentobarbital (3.0 mM)	329.7 ± 64.8†	145.5 ± 18.4‡
Aprobarbital (3.0 mM)	71.6 ± 12.3	64.9 ± 4.5§

* The values of K_m and V_{max} (mean ± S.E.M.) were obtained by weighted regression analysis [14] of double-reciprocal plots of the GABA uptake data obtained at GABA concentrations of 1, 5, 15, 50, 100 and 250 μM (N = 4–5). The GABA uptake had been corrected for the non-saturable component of the uptake [1]. The uncertainty of this correction is not included in the statistical treatment. It is of negligible importance for the determination of K_m but of considerable importance for the determination of V_{max} at the high K_m observed in the presence of pentobarbital. This probably explains the apparent increase in V_{max} under these conditions. For further details see methods.

† $P < 0.005$.

‡ $P < 0.05$.

§ $P < 0.001$.

such as glial GABA uptake sites. Both at the uptake site (present work) and at least some other sites [18] the anticonvulsant barbiturate phenobarbital seems less potent than the hypnotic and anesthetic barbiturate pentobarbital, suggesting that these effects are not specifically correlated with the antiepileptic properties of barbiturates.

GABA uptake into astrocytes in primary cultures was inhibited by both pentobarbital and aprobarbital, but the former barbiturate exerted a competitive inhibition and the latter a non-competitive inhibition. The effect of pentobarbital was observed at a pharmacologically relevant concentration.

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