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Influence of Carbamazepine and Phenytoin on Spontaneous Activity of Cerebellar Neurons

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Summary

Action of carbamazepine (50 mg/kg i.p.) and phenytoin (60 mg/kg i.p.) on the activity of cerebellar neurones was studied in rats under urethane anaesthesia. Carbamazepine markedly decreased the firing frequency of all ten neurones recorded continually before and after drug administration. The same conclusion was reached when a group of 53 cells recorded before drug administration was compared with 48 neurones recorded after carbamazepine administration only. The effects of phenytoin were ambiguous – a decrease as well as an increase in frequency was recorded. The solvent used did not change cerebellar unit activity. Cerebellum cannot be considered as a possible target structure for phenytoin but it might be a target for carbamazepine action.

Key words

Rat – Cerebellar neurones – Carbamazepine – Phenytoin

Introduction

Mechanisms of action of antiepileptic drugs (AEDs) are being extensively studied at the molecular level. It is possible to discriminate at least two basic types of action of AEDs: 1. action on ion channels, i.e. on the cellular membrane, 2. interaction with receptors.

Phenytoin and carbamazepine might serve as examples of AEDs directly influencing the cellular membrane, especially Na^+ channels (DeLorenzo 1989, Macdonald 1989).

Due to the focusing of research on the molecular mechanisms, less attention has been paid to the structural basis of the action of AEDs. The action of an AED might be exerted only in a small region (or in some parts) of the central nervous system as was shown by Gale (1989) for vigabatrin. This drug exerts its action in the midbrain, whereas injections of vigabatrin into the caudate, thalamus, superior colliculus and pontine regions are ineffective. Julien and Halpern (1972) hypothesized that phenytoin primarily affects cerebellar neurones and thus exhibits its anticonvulsant action through the inhibitory output of the cerebellum. Similarly, Julien suggested the cerebellum as a target structure for carbamazepine (Julien and Hollister 1975, Julien 1982). The estimation of the structure where the AEDs exhibit their actions is of primary importance for molecular neurobiology in order to determine which structure is to be studied.

These data led us to study the changes of the activity of cerebellar neurones under the influence of the two AEDs mentioned above to verify if the cerebellum might be considered as a target for phenytoin and carbamazepine.

Materials and Methods

The unit activity was recorded extracellularly in cerebellar cortical neurones in 37 male Wistar rats. Anaesthesia was induced by an intraperitoneal injection of urethane (1.5 g/kg). A small craniotomy was made (AP 9.6 – 10.6 mm from the bregma along the midline, so that lobuli V and VI of the cerebellar vermis were accessible) and the dura mater was carefully removed. The rat was fixed in a stereotaxic apparatus and its body temperature was maintained by an electrically heated pad.

Table 1
Survey of the registered neurones

Type of activity		Number of neurones
Uninfluenced activity only		53
Activity only after administration of	CBZ	48
	PHT	23
Activity before and after administration of	CBZ	10
	CBZ	10
	PHT	10
	Solvent	10

Activity of neurones in the cerebellar cortex was registered by glass micropipettes filled with 3 M KCl, with a resistance from 5 to 15 MΩ. A total of 154 neurones were recorded in the depth from 100 to 3000 μm. We always tried to have the recording from the same neurone before as well as after drug administration. Such continual recordings were obtained in 30 neurones (Table 1). In addition, recordings were obtained from 53 uninfluenced neurones and from another 71 neurones influenced by the drugs (Table 1). Because the records of all neurones influenced by CBZ and/or PHT were made between 20 and 90 min after drug application, plasma half-life being more than 3 h for PHT (Woodbury 1989) and more than 1.5 h for CBZ (Faigle and Feldmann 1989), an effective level of both drugs in the organism was maintained during that time (Marešová *et al.* 1991).

The influence of carbamazepine (CBZ, Sigma), phenytoin (PHT, Epanutin^R, Parke and Davis), and a solvent of both drugs (mixture of propyleneglycol, ethanol and water in the ratio of 5:2:3) was tested. CBZ (freshly dissolved in the concentration of 25 mg/ml) was administered in the dose of 25 mg/kg i.p. in 13 animals, PHT in the dose of 60 mg/kg i.p. in 14 animals, the solvent (dose of 1 ml/kg) in 10 animals.

Unit activity was recorded on magnetic tape after preamplification and discrimination as unified rectangular pulses. The number of spikes at 10-second intervals was counted by means of a simple microprocessor. Frequencies of neuronal activity obtained were processed by non-parametric variant of analysis of variance (Kruskall-Wallis test and associated multiple comparisons between groups). $P<0.05$ level of significance was used in all tests. The results of data processing were presented in the form of bar charts, Notched Box and Whisker Plots and linear regression lines. The type of statistical distribution was tested before processing and it was found that the logarithms of frequencies fitted the normal distribution reasonably well.

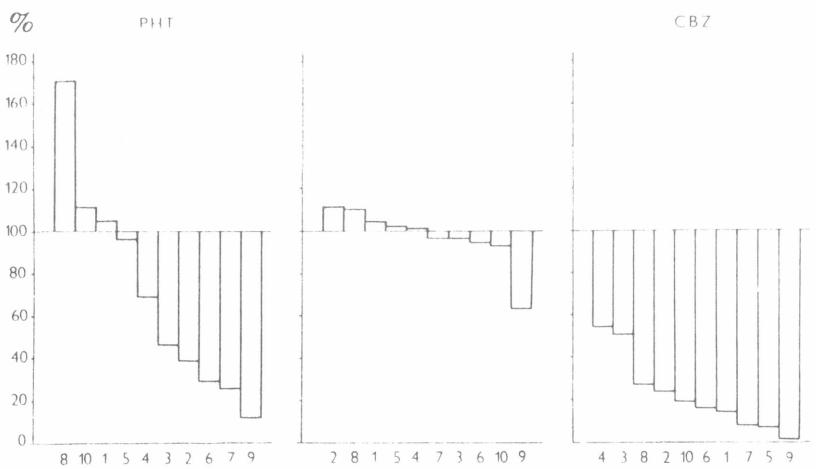

Fig. 1
Frequency changes in the activity of cerebellar neurones caused by phenytoin (PHT, left), solvent (S, middle) and carbamazepine (CBZ, right). The data for 10 neurones for each substance are presented. Abscissa – serial numbers of neurones measured. Ordinate – relative changes of the frequency (spontaneous frequency before drug administration is taken as 100%).

Results

Neurones from which activity was recorded both before and after the administration of CBZ as well as PHT exhibited changes of firing frequency (Fig. 1).

CBZ diminished the firing frequencies of all 10 cerebellar neurones recorded continually. Linear regression (for CBZ) disclosed the existence of a linear dependence of the frequency changes evoked by CBZ administration on the background frequencies, i.e. the higher was the frequency before CBZ injection, the higher the effect was caused by the injection (Fig. 2). Therefore, the relative frequency changes were considered to be more convenient for estimating the CBZ influence than the absolute values.

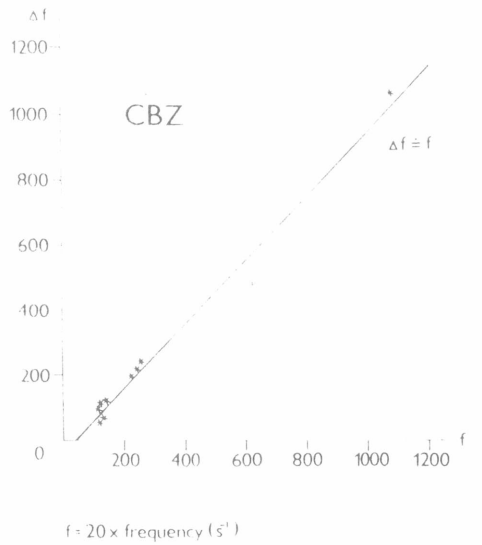

Fig. 2
Dependence of the frequency changes after carbamazepine on the background pre-drug frequency. Data from 10 neurones are presented. Each asterisk represents one neurone. Abscissa – the frequency before CBZ administration. Ordinate – the difference between frequency before and after CBZ administration.

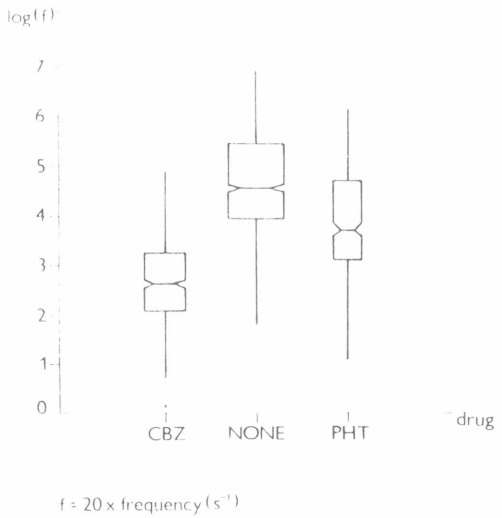

Fig. 3
Influence of CBZ and PHT on unit activity. A notched box-and-whisker plot is a modification of the standard box-and-whisker plot. A notched is added to each box corresponding to the width of a confidence interval for the median, while the width of the box is proportional to the square root of the number of observations in the data set. Extreme points beyond 1.5 times the box length (interquartile range) are plotted as individual adjacent values. Abscissa – the drug injected. Ordinate – logarithms of frequencies (Hz).

The results with PHT were not homogeneous. PHT depressed the frequency of unit activity in seven cases (very strongly in five of them). On the other hand, the frequency in the remaining three neurones increased, in one of them markedly (Fig. 1).

The solvent left the frequency of unit activity practically unchanged. The differences between the frequencies before and after solvent administration were moderate in all but one neurone, where a reduction of 37 % was observed. The positive and negative changes were seen in the same number of cases (Fig. 1).

If the continuous recordings were combined with those where the activity was registered only before or after drug administration (i.e. a total of 83 cells recorded before drug administration, 58 neurones after CBZ administration, and 33 neurones after PHT administration), the same changes as mentioned above could be demonstrated (Fig. 3). The CBZ administration significantly decreased the frequencies of cerebellar neurones. The changes induced by PHT were in the same direction but were not statistically significant (Fig. 3).

Discussion

Our results demonstrating a marked depressant effect of CBZ on the activity of cerebellar neurones are different from those of Julien and Hollister (1975). Even the increase in firing frequency after PHT described by Julien and Halpern (1972) was found only exceptionally in our experiments. We did not identify the recorded neurones, but there is a high probability that we recorded from Purkyně cells as the largest neurones in the cerebellar cortex. Even in the case that Purkyně neurones form only a part of the recorded cell population, it is impossible to confirm the hypothesis of Julien that phenytoin and carbamazepine exert their anticonvulsant action by enhancing the activity of Purkyně cells in the cerebellum. If we accept the high probability that we recorded from Purkyně cells, the decrease in firing frequency of cerebellar neurones might be feasibly connected with an anticonvulsant effect. The majority of these cells send axons to deep cerebellar nuclei, which represent an output from the cerebellum. Both Purkyně cells and cells of the deep nuclei are inhibitory neurones (Ito *et al.* 1964, 1970). Thus, a decrease in the activity of Purkyně cells might represent disinhibition of neurones in cerebellar nuclei and an increased inhibitory influence of the cerebellum on other parts of the central nervous system. Other explanations are also possible, since we cannot exclude an indirect effect and therefore further analysis is necessary. In any case, the cerebellum cannot be considered as the main target structure for the action of phenytoin. The possibility

that CBZ exerts its anticonvulsant action by means of cerebellar mechanisms, must be studied further.

The mechanism of carbamazepine action is not fully known, but there are data available demonstrating a suppressant effect on high frequency firing of nerve cells in culture (Macdonald and McLean 1986). This same action is taken as an expression of binding to sodium channels and enhancement of voltage-dependent sodium channel inactivation (Macdonald 1989). The same action has also been described for phenytoin, but many other actions are known for this drug (for review see Woodbury and Kemp 1971, Woodbury 1982, DeLorenzo 1989). The variable effect on cerebellar cells might be an expression of the interaction of various mechanisms of phenytoin action. On the other hand, there are also data suggesting another mechanism of action of carbamazepine – competitive inhibition of adenosine binding (Marangos *et al.* 1983). The difference found between the action of CBZ and that of PHT in this study as well as in some other experiments (Mareš *et al.* 1993) speaks against a similar mechanism of action of these two drugs.

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