Physiol. Res. 43: 57–60, 1994

RAPID COMMUNICATION

Influence of Flunarizine on Hippocampal Epileptic Afterdischarges in the Rat

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Received June 7, 1993
Accepted September 20, 1993

Summary

Epileptic afterdischarges elicited by electrical stimulation of the dorsal hippocampus in freely moving rats were not significantly changed by flunarizine administration in comparison with control sessions in which the animals received the solvent only. On the other hand, flunarizine significantly reduced the number of wet dog shakes, the main automatisms accompanying limbic afterdischarges.

Key words

Epileptic afterdischarges – Hippocampus – Rat – Flunarizine

Complex partial seizures represent the majority of seizures refractory to contemporaneous pharmacotherapy (Gates and Gumnit 1990). Therefore a search for new potential antiepileptics is mainly directed to drugs effective against this type of seizures. A calcium overflow blocker, flunarizine, has been tried successfully as an add-on therapy (in majority of cases against complex partial seizures – Overweg *et al.* 1984, Binnie *et al.* 1985). Clinical trials of flunarizine have been based on its anticonvulsant activity in various animal models of epileptic seizures: generalized tonic-clonic seizures elicited by electroshock, pentylenetetrazol or bicuculline (Desmedt *et al.* 1975), reflex seizures (de Sarro *et al.* 1986) or tonic forepaw seizures in the bicuculline seizure threshold test (Wauquier *et al.* 1986). We have demonstrated a specific action against pentylenetetrazol-induced generalized tonic-clonic seizures (leaving untouched minimal clonic seizures) in adult as well as in immature rats (Pohl and Mareš 1987). To delineate further the anticonvulsant profile of flunarizine, we started to study the effect of flunarizine against a model of complex partial seizures – epileptic afterdischarges (ADs) elicited by electrical stimulation of the hippocampus in rats. This model has been used in our laboratory for testing classical as well as potential antiepileptic drugs (Velíšek and Mareš 1987, Mikolášová *et al.* – submitted).

Seven male adult albino rats of the Wistar strain were surgically prepared under pentobarbital anaesthesia (60 mg/kg i.p.). Stimulation electrodes were stereotaxically introduced into the right dorsal hippocampus at coordinates AP=3.5; L=3.5; H=3 mm from the cortical surface according to Fifková and Maršala (1960). The third wire serving as a recording electrode was attached to the two stimulating electrodes. All three wires (with a diameter of 0.1 mm) twisted together were isolated up to their tips and these tips were about 50 μm in diameter. The distance between the tips of two stimulation electrodes was thus 0.1 mm. Silver ball cortical recording electrodes were placed over sensorimotor (AP=0; L=2.5 mm) and visual (AP=6; L=4 mm) areas of both hemispheres, an indifferent electrode was localized on the nasal bone. The electrodes were attached to the connector and the whole assembly was cemented to the skull by a fast curing dental acrylic.

The experiments started after a recovery period of one week. The hippocampus was stimulated by 15 s series of rectangular pulses of 1 ms duration at a frequency of 8 Hz. The intensity equal to double the threshold value necessary for eliciting of an evoked potential in the sensorimotor cortex was used.

Stimulation was repeated four times in each session. The intervals between the end of an afterdischarge and the beginning of the next stimulation were 15 min. Each animal underwent three sessions: during the first one the intensity of stimulation was established; the second and third sessions were experimental, three rats were subjected to the control stimulations with administration of the solvent in the second and of flunarizine in the third session, In the remaining four animals the experimental procedure was reversed.

Flunarizine (a generous gift from Janssen Pharmaceutica) was administered intraperitoneally as a freshly prepared solution in a mixture of propyleneglycol, ethanol and water (ratio 5:2:3) in concentration of 20 mg/ml. Flunarizine in the dose of 20 mg/kg was injected immediately after the end of the first afterdischarge, so that this first AD served as the control. In the control sessions the animals were given the corresponding amount of the solvent (1 ml/kg i.p.). The criterion used for inclusion of the animals into this study was the presence of an afterdischarge of the "limbic" type (see below).

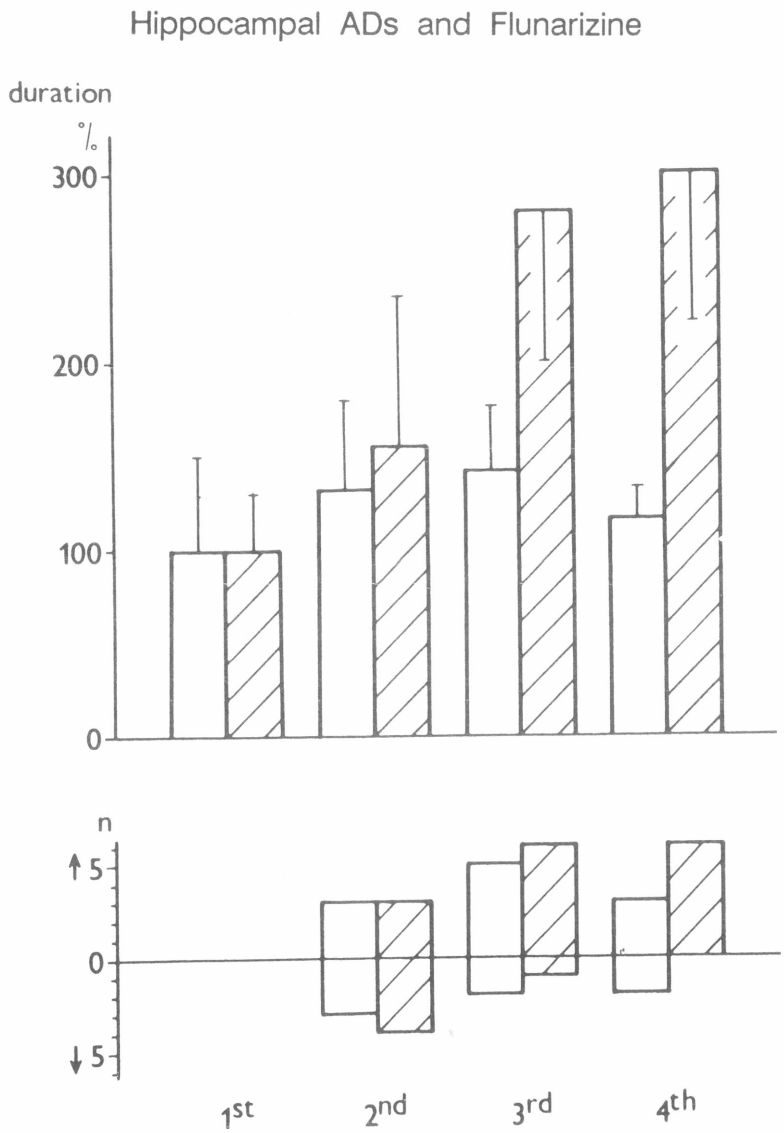

Fig. 1
Relative duration of hippocampal afterdischarges (upper part, mean ±S.E.M.) and number of animals exhibiting changes in duration of the second to fourth afterdischarges in comparison to the first one. In both parts – white columns = animals received solvent injection; hatched columns = animals injected flunarizine. Abscissa: the first to the fourth afterdischarge; upper ordinate: relative duration of afterdischarges, the duration of the first, predrug or presolvent AD is taken as 100 %; lower ordinate: absolute number af animals in which the second, third and fourths ADs are longer (upwards) or shorter (downwards) than the first one. Cases when the change was less than 10 % are not included in the Figure.

The duration of ADs was measured from the EEG recordings and the most conspicuous behavioural correlates of ADs – wet dog shakes – were counted. Both these phenomena were statistically evaluated by means of an analysis of variance. The level of significance was set at 5 %.

All rats exhibited epileptic afterdischarges in both control and drug sessions. In the control sessions, the EEG pattern of ADs was formed by fast spikes of low amplitude or huge delta waves with superimposed fast spike activity. With the stimulation intensity used there were no differences in duration and shape of ADs recorded in the hippocampus and neocortex. Behavioural correlates – wet dog shakes (WDS) appeared towards the end of ADs and were present for a short time (10–15 s) after the ADs had stopped. Approximately one minute after the end of the AD, a recurrent AD appeared; its EEG pattern was always simpler than that of the stimulus-bound AD. It was usually formed by sharp delta or theta waves. No change in behavior was observed at the beginning of recurrent ADs. WDS reappeared again towards the end of recurrent ADs and lasted for some seconds after the EEG had become free of epileptic graphoelements. Repeated stimulations led to a moderate tendency to prolongation of ADs in animals under control conditions. Similar progressive prolongation of recurrent ADs reached the level of statistical significance. Even the number of WDS increased with repeated ADs.

Flunarizine did not change the EEG pattern of afterdischarges. It augmented the lengthening of ADs so that the significant linear trend was proved statistically and the duration of the third and fourth ADs was significantly longer than that of the first (predrug) AD. The fourth AD was longer than the first one in six rats, the remaining animal exhibited the first and fourth ADs of the same duration. Recurrent ADs again exhibited a progressive significant prolongation with repeated stimulations. Comparison with animals receiving only solvent did not demonstrate any significant change in ADs as well as in recurrent ADs. Number of WDS decreased after flunarizine from the control value of 3.1±0.6 (mean±S.E.M.) to values between 1.2 and 1.6, the level of statistical significance was reached for the second and fourth ADs. Solvent did not change this parameter statistically.

Flunarizine was unable to suppress the hippocampal afterdischarges in our experimental paradigm. On the contrary, the tendency to prolongation became statistically significant. On the other hand, the significant suppression of wet dog shakes, behavioural automatisms accompanying this type of epileptic ADs, proved the anticonvulsant effect of flunarizine. This effect might be realized in different ways: 1. blockade of the spread of epileptic activity from the hippocampus to the generator of WDS; 2. suppression of the hypothetical generator of WDS. Inhibition of the locomotor apparatus necessary for movements forming the motor pattern of WDS is highly improbable because of a complete lack of findings demonstrating a marked effect of flunarizine on neuromuscular transmission or directly on muscles and normal locomotion of our animals after flunarizine injection. Wet dog shakes could be elicited in many ways: by electrical stimulation of limbic structures inducing epileptic afterdischarges (Racine and Burnham 1984), by systemic or intracerebroventricular injections of kainic acid (Ben-Ari 1985), by morphine withdrawal (Redmond and Krystal 1984) or by serotonergic mechanisms (Beddard and Pycock 1977). Unfortunately, there are no data about the localization of the generator of WDS, so that it is impossible to decide which of the two explanations is correct.

Our results demonstrated contraversial effects of flunarizine in the model used. Flunarizine probably affects the generator of epileptic afterdischarges in the hippocampus facilitating the genesis of ADs or at least it does not interfere with the generation of ADs. The possible mechanism of the progressive lengthening of ADs seen in the solvent as well as the flunarizine group cannot be explained at present. The protective effects of flunarizine (Wauquier 1982) might play a role allowing epileptic activity to be sustained for a longer time than under control conditions. On the other hand, the activation of motor areas generating wet dog shakes is restricted. Our results together with other data on the anticonvulsant action of this calcium channel blocker emphasize the need of further studies delineating the anticonvulsant profile of flunarizine. This drug might be used as an add-on therapy of epilepsy only after further extensive studies.

Acknowledgement

This study was supported by grants No. 71107 of the Czechoslovak Academy of Sciences and No. 0631-3 of the Czech Ministry of Health. The authors would like to express their thanks to Dr. J. Vorlíček from the Department of Biomathematics, Institute of Physiology, Academy of Sciences of the Czech Republic for valuable help in the statistical evaluation of the results.

References

BEDDARD P., PYCOCK C.J.: "Wet-dog" behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity. *Neuropharmacology* **16:** 663–670, 1977.

BEN-ARI Y.: Limbic seizure and brain damage produced by kainic acid: Mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* **14:** 375–403, 1985.

BINNIE C.D., DEBEUKELAR F., MEIJER J.W.A., MEINARDI H., OVERWEG J., WAUQUIER A., van WIERINGEN A.: Open dose-ranging trial of flunarizine as add-on therapy in epilepsy. *Epilepsia* **26:** 424–428, 1985.

DE SARRO G.B., NISTICÓ G., MELDRUM B.: Anticonvulsant properties of flunarizine on reflex and generalized models of epilepsy. *Neuropharmacology* **25:** 695–701, 1986.

DESMEDT L.K.C., NIEMEGEERS C.J.E., JANSSEN P.A.J.: Anticonvulsant properties of cinarizine and flunarizine in rats and mice. *Arzneimittelforsch.* **25:** 1408–1413, 1975.

FIFKOVÁ E., MARŠALA J.: Stereotaxic Atlas of the Brain of Rabbit, Rat and Cat. (In Czech). Státní zdravotnické nakladatelství, Prague 1960.

GATES J.R., GUMNIT R.J.: Partial seizures of temporal lobe origin. In: *Comprehensive Epileptology,* M. DAM, L. GRAM (eds), Raven Press, New York 1990, pp. 187–195.

OVERWEG J., BINNIE C.D., MEIJER J.W.A., MEINARDI H., NUIJTEN S.T.M., SCHMALTZ S., WAUQUIER A.: Double-blind placebo-conotrolled trial of flunarizine as add-on therapy in epilepsy. *Epilepsia* **25:** 217–222, 1984.

POHL M., MAREŠ P.: Flunarizine influences metrazol-induced seizures in developing rats. *Epilepsy Res.* **1:** 302–305, 1987.

RACINE R.J., BURNHAM W.M.: The kindling model. In: *Electrophysiology of Epilepsy,* P.A. SCHWARTZKROIN, H.V. WHEAL (eds), Academic Press, London, 1984, pp. 153–171.

REDMOND D.E., Jr., KRYSTAL J.H.: Multiple mechanisms of withdrawal from opioid drugs. *Annu. Rev. Neurosci.* **7:** 443–478, 1984.

VELÍŠEK L., MAREŠ P.: Influence of antiepileptic drugs on hippocampal afterdischarges. *Neuroscience* **22:** Suppl. S666, 1987.

WAUQUIER A.: Brain protective properties of etomidate and flunarizine. *J. Cerebr. Blood Flow Metabol.* **2:** (Suppl. 1) 553–556, 1982.

WAUQUIER A., ASHTON D., CLINCKE G., FRANSEN J., GILLARDIN J.M., JANSSEN P.A.J.: Anticonvulsant profile of flunarizine. *Drug Dev. Res.* **7:** 49–60, 1986.

Reprint Requests

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