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FULL-LENGTH ORIGINAL RESEARCH

Protein kinase inhibitor as a potential candidate for epilepsy treatment

***Zita Gajda, *Rita Török, †Zoltán Horváth, †Csaba Szántai-Kis, †‡László Őrfi, †§György Kéri, and *Magdolna Szente**

***Department of Comparative Physiology, Anatomy and Neuroscience, University of Szeged, Szeged, Hungary; †Vichem Chemie LTD, Budapest, Hungary; ‡Department of Pharmaceutical Chemistry, Semmelweis University, Budapest, Hungary; and §Pathobiochemistry Research Group of Hungarian Academy of Sciences, Department of Medical Chemistry, Semmelweis University, Budapest, Hungary**

Summary

Purpose: Effects of the "VID-82925" kinase inhibitor molecule were investigated both during the developing phase as well as during the stable phase of the focus with spontaneous recurrent seizures using the 4-AP-induced in vivo epilepsy model in anesthetized rats.

Methods: In electrophysiologic experiments, VID-82925 (0.85 mg/kg) was injected intravenously either before the induction (pretreatment) or after the development of the stable focus (treatment). Reference drugs carbamazepine (4.8 mg/kg) and levetiracetam (50 mg/kg) were employed using the same experimental paradigm. The antiepileptic effect of VID-82925 was also compared to those of the broad-spectrum gap junction channel blocker carbenoxolone (10 mM).

Key Findings: Pretreatment with VID-82925 revealed an antiepileptogenic effect as it suppressed significantly the manifestation of the epileptiform activity not only during the developing phase, but also for a considerable long period during the stable phase of the focus. The current data do not allow us to differentiate an antiictal treatment effect from an antiepileptogenic effect of the compound during the stable phase of the focus. Treatment with VID-82925 was also effective against ictogenesis during the stable phase of the focus. Pretreatment with levetiracetam failed to exert any antiepileptogenic effect. The antiepileptic effects of VID-82925 and of the reference drugs on the epileptiform activity of the stable focus were comparable in intensity; however, the effect of VID-82925 was 2–3 times longer. The effects of VID-82925 and of carbenoxolone overlapped one another to some extent, suggesting that VID-82925 may exert its effects at least partially through blocking of gap junctional communication.

Significance: Our results indicate that inhibition of protein kinases may also provide an effective strategy for the development of a drug that is not only antiepileptic but also depresses the course of epileptogenesis.

KEY WORDS: Antiepileptogenic, Antiepileptic, Kinase inhibitor, In vivo.

According to the World Health Organization (WHO), approximately 50 million people in the world have epilepsy. The currently available antiepileptic drugs (AEDs) although effective as suppressants of ictogenesis (i.e., manifestation and spread of seizures in the already epileptic brain (Loscher & Schmidt, 2006), do not affect the development of epilepsy (Dudek et al., 2008). In addition, despite progress in understanding the pathogenesis of epilepsy, seizures remain uncontrolled in at least 20–30% of patients. In general, these drugs act either by the modulation of voltage-gated ion channels or by enhancement of synaptic inhibition mediated by γ-aminobutyric acid $(GABA)_A$ receptors or by inhibition of synaptic excitation mediated by ionotropic glutamate receptors (Rogawski, 2006).

The significant number of patients with poorly controlled seizures suggests that some clinically relevant proepileptic mechanisms are not targeted by any of the currently available AEDs. The reason for this could be that most of the AEDs were tested by using the same few classical epilepsy models (Pitkanen et al., 2007; Smith et al., 2007; Dudek et al., 2008), which mainly involve the similar actions, without consideration of the variations in the pathophysiologic mechanisms that result in epilepsy. Therefore, to search for possible new cellular and/or molecular targets in combination with the use of new animal models may provide more successful strategies for the development of drugs that might be effective for the currently drug-resistant epilepsies, as well as for preventing epileptogenesis.

The nonsynaptic intercellular communication via gap junction (GJ) channels both between inhibitory interneurons and astrocytes have been shown recently to be an efficient

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Address correspondence to Professor Magdolna Szente, Ph.D., D.Sc., Department of Comparative Physiology, Anatomy and Neuroscience, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary.
E-mail: szente@bio.u-szeged.hu

synchronizing mechanism (Tamas et al., 2000; Giaume et al., 2010) that contributes to the manifestation and propagation of seizures and to the enhanced epileptogenicity of the adult (Perez Velazquez & Carlen, 2000; Szente et al., 2002; Gajda et al., 2003, 2005) and developing neocortex (Gajda et al., 2006 and see for review Szente, 2008). Growing evidence demonstrates that protein phosphorylation by various kinases are involved in the regulation of GJ communication (Cruciani & Mikalsen, 2002; Moreno & Lau, 2007; Solan & Lampe, 2009).

Therefore, in a series of experiments, we tested the effects of some kinase inhibitors on the semichronic 4-aminopyridine (4-AP)–induced in vivo epilepsy model both during the epileptogenesis as well as during the stable phase of the focus with spontaneous recurrent seizures. Because the consecutive spontaneous seizures appear with short interseizure intervals in this model, the possibility of long seizure monitoring provide good opportunities to carry out reliable quantitative measurements on the effects of pharmacologic agents on the electrophysiologic manifestation of seizures. In the present paper we draw attention to the ''VID-82925'' marked molecule (provided by Vichem Chemie Ltd, Budapest, Hungary), which turned out to be one of the most efficient kinase inhibitors against epileptiform activity.

In the 4-AP epilepsy model, two phases of the maturation of the epileptic focus can be distinguished. The *developing phase* of the focus takes approximately 50 ± 10 min that includes the latency period (usually 1–3 min), elapsing between the application of the 4-AP and the appearance of the first seizure (Fig. 2A, control in light shaded panel), and the subsequent period when the frequency and the duration of the seizures as well as the amplitudes of seizure discharges increase gradually up to a stable level. During the following *stable phase* of the epileptic focus, the parameters of the epileptiform activity remain rather reliable for a considerable time period (Fig. 2A, control in dark shaded panel). Of course it is not possible to delineate exactly a sharp border between the developing phase and the stabilized phase of the focus. Since epileptogenesis is the gradual process whereby normal brain is transformed into a state susceptible to spontaneous, episodic, time-limited, recurrent seizures through the initiation and maturation of an epileptogenic focus (Engel et al., 2008), in our experimental circumstances, the developing phase of the focus might be considered as epileptogenesis.

Our earlier observations (Szente et al., 2002; Gajda et al., 2005, 2006) suggest that different cellular and network mechanisms could be involved in the development of the epileptic focus and in the maintenance of seizure activity of an already stable epileptic focus. Therefore, in the present study we aimed to investigate the effects of VID-82925 both during the developing phase of the epileptic focus and during the activity of the already stable epileptic focus. With the intention of estimating the power of the effects of VID-82925 on epileptiform activity, its data were compared with those of the well-known and clinically widely used AEDs (carbamazepine and levetiracetam) that were also tested on the 4-AP epilepsy model in identical circumstances. Previous experiments in the kindling model suggested that levetiracetam in addition to its seizure-suppressing activity, may possess antiepileptogenic activity (Löscher et al., 1998). Attempting to check the possible blocking effect of VID-82925 on GJal communication, we tested by electrophysiologic technique whether the effects of VID-82925 and of the broad-spectrum GJ blocker carbenoxolone overlap fully or partially with each other.

Methods

Experimental procedures

Acute electrophysiologic experiments were carried out on adult (30–40-day-old) Wistar rats (body weight: 200–300 g) of either gender (Charles River Laboratories, Budapest, Hungary). All experimental procedures were conducted in accordance with the United States Public Health Service's Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of Szeged.

Under transient diethyl ether narcosis, a metal cannula was inserted into the tail marginal vein, and then the animals were anesthetized intravenously (i.v.) with sodium pentobarbital (50 mg/kg). The trachea was cannulated with a polyethylene tube. The heads of the animals were secured in a stereotaxic instrument. For recording of electrocortical activity (ECoG), four holes (2–3 mm wide) were drilled in the skull, and the dura mater was carefully removed at the prospective site of the primary focus (Pf). Then animals were left to rest for 30 min.

The seizure activity at the Pf was induced by the local application of the potassium channel blocker 4-AP (on a 3 × 3 mm piece of filter paper soaked with saline) to the somatosensory cortical surface (Szente & Baranyi, 1987; Szente et al., 2002) in both female (n = 3) and male (n = 3) rats. Because no significant differences were found in the results of male and female rats, data were pooled together for statistical analysis. Four silver ball electrodes connected to an eight-channel electroencephalograph were used to record ECoG from the Pf and mirror focus (Mf, the homotopic area contralateral to the Pf), as well as from two posterior cortical points, in order to detect the manifestation and propagation of epileptiform discharges. In parallel with ECoG monitoring, electrocardiography (ECG) was also performed during the experiments to test the possible effect of the tested drugs on the heart activity.

The general state of the animals (level of anaesthesia, body temperature) was checked regularly throughout the experiments. All efforts were made to minimize the number of animals used and their suffering. At the end of the

experiments, the animals were given a lethal dose of sodium pentobarbital.

Tested drugs

VID-82925 (0.85 mg/kg) was dissolved in 0.9% saline and sonicated for 45 min for complete dissolving. Carbenoxolone (10 mM; Sigma Aldrich, Budapest, Hungary) was dissolved in 0.9% saline. Levetiracetam (Keppra, 50 mg/kg; provided by the Pharmacy of Faculty of Medicine of University of Szeged) was in the form of concentrated solution and diluted with saline the day of the experiments. For choosing the appropriate dose of levetiracetam for rats, we considered its dosage in the clinical practice described in the literature. Levetiracetam is used from 10 up to 40 mg/kg/day for children and from 1,000 up to 3,000 mg/day for adults (Toublanc et al., 2008). For carbamazepine (4.8 mg/kg; Sigma Aldrich) first stock solution was prepared in 100% DMSO (Sigma Aldrich) and then diluted with saline to appropriate concentrations for the individual experiments. Final DMSO concentration was 1%. This concentration of DMSO has been shown to have no effects on neuronal excitability (Mclean & Macdonald, 1986).

Drug testing protocols

Two experimental protocols were used in which all tested drugs except carbenoxolone were administered intravenously. The mechanisms of actions of the tested AEDs and of pentobarbital are different (Schachter, 2007; Dudek et al., 2008); however, their possible interaction cannot be excluded. In any case, we paid attention that all of the tested chemicals were investigated in identical experimental paradigms. In the *pretreatment experiments*, we aimed to study the effects of tested drugs on the development of the epileptic focus, which means both the premanipulation of the cortex by the tested drug before 4-AP application (Fig. 2A, dotted panel), as well as the manipulation of the cortex during the developing phase of the epileptic focus (Fig. 2A, light shaded panel). In *treatment experiments*, we aimed to study the effects of the tested drugs on the ictogenesis; therefore, the drugs were applied during the stable phase of the focus producing spontaneous recurrent seizures (Fig. 2A, dark shaded panel).

Pretreatment

The rats were injected by VID-82925 (i.v.; n = 10) or by levetiracetam (i.v.; n = 5), respectively, prior to the application of 4-AP. The time between the application of the drugs and the application of 4-AP (60 min in the case of VID-82925 and 20 min in the case of levetiracetam) was chosen according to the peak time of the drug's effect (observed in treatment experiments) to be sure that the drug was still effective during the entire developing phase. Data were recorded until the effects of the drug started to decline but at least for 220 min after the application of 4-AP. The control data were collected from a separate group of rats (n = 6) submitted to an identical experimental paradigm, but pretreatment was carried out with saline instead of the drugs.

Treatment

The rats were treated by VID-82925 (iv; n = 10), by carbamazepine (i.v.; n = 6) or by levetiracetam (i.v.; n = 5), or by carbenoxolone (locally by means of a 3 × 3 mm piece of filter paper, soaked with carbenoxolone solution applied on top of the filter paper containing 4-AP), respectively, 60 min after the application of 4-AP (35–40 seizures/60 min). Data were collected until the effects of the drug started to decline but at least for 260 min after the application of the drug. In these experiments, the data collected from the same animal for 20 min before the application of the drug served as the control values.

Checking the possible blocking effect of VID-82925 on GJal communication

In one series of experiments, after 35–40 spontaneously repeated seizures, the Pf was first treated with carbenoxolone; then before development of its peak effects (10 min after the application of carbenoxolone), VID-82925 was injected (n = 10). The data were then collected for an additional 250 min.

In the other series of experiments, after 35–40 spontaneously repeated seizures, the VID-82925 was injected first, then before development of its maximum effects (100 min after the administration of VID-82925), carbenoxolone was applied (n = 10). The data were then collected for an additional 160 min.

Statistical analysis

In addition to ECoG paper recording, data were also stored in a computer memory with the aid of Digidata 1200B, and analyzed by Mathcad (MathSoft, Cambridge, MA, U.S.A.), Origin (OriginLab, Northampton, MA, U.S.A.) processing software completed by a homemade statistical program after the experiments. The effects of the drugs on epileptiform activity were assessed by measuring the latency of the first seizure (the time that elapses between the application of 4-AP and the appearance of the first seizure), the number and duration of seizures as well as the amplitude of epileptiform discharges of Pf. The summated ictal activity is a calculated parameter and indicates the total duration of seizure activity during a certain time window; therefore, it is useful for evaluating the intensity of epileptiform activity. The summated ictal activity was determined by multiplying the number of individual seizures by their durations measured in 20 min time periods.

Results are given as means ± SD. Student's *t*-test was used to assess significant differences between the control and experimental groups of ECoG and ECG data. The level of statistical significance was set at $p < 0.05$.

Results

Depressing effect of VID-82925 on the development of the epileptic focus

The VID-82925 did not noticeably influence the basic electrocortical activity (Fig. 1A). Although the latency of the first seizure (138 ± 42 s) was in the same range in the presence of VID-82925 as in controls (139 ± 52 s), pretreatment with VID-82925 revealed a strong antiepileptogenic effect as suppressed significantly the manifestation of the epileptiform activity not only during the developing phase, but also for a considerably long period during the stable phase of the focus.

The number of seizures was only 65% of the control value at the 20th min after the induction of epileptiform activity, and then reached its lowest level (24.6% of the control value) at the 120th min (Table 1). From here it increased but was still only 63% of the control value at the 220th min. The durations of seizures were slightly shorter than the controls values until the 80th min (Table 1). By the 100th min it approached the control level. From here seizures gradually became longer and the duration was 134% of the control value at the 220th min. Despite the increase in seizure duration the overall effects of VID-82925 are depressing because of the prominent decrease of the number of seizures. The summated ictal activity was only 55% of the control value at the 20th min; by the 40th min reached its minimum (31% of the control value) and then stayed persistently at considerably decreased level until the 180th min (Fig. 2A). At the 200th min (260 min after the administration of VID-82925) it gradually approached the control level.

The amplitudes of seizure discharges were only 81% of the control value at the 20th min and only 73% at the 80th min (Table 1). From here the difference between the pretreated and control animals decreased, but the amplitudes

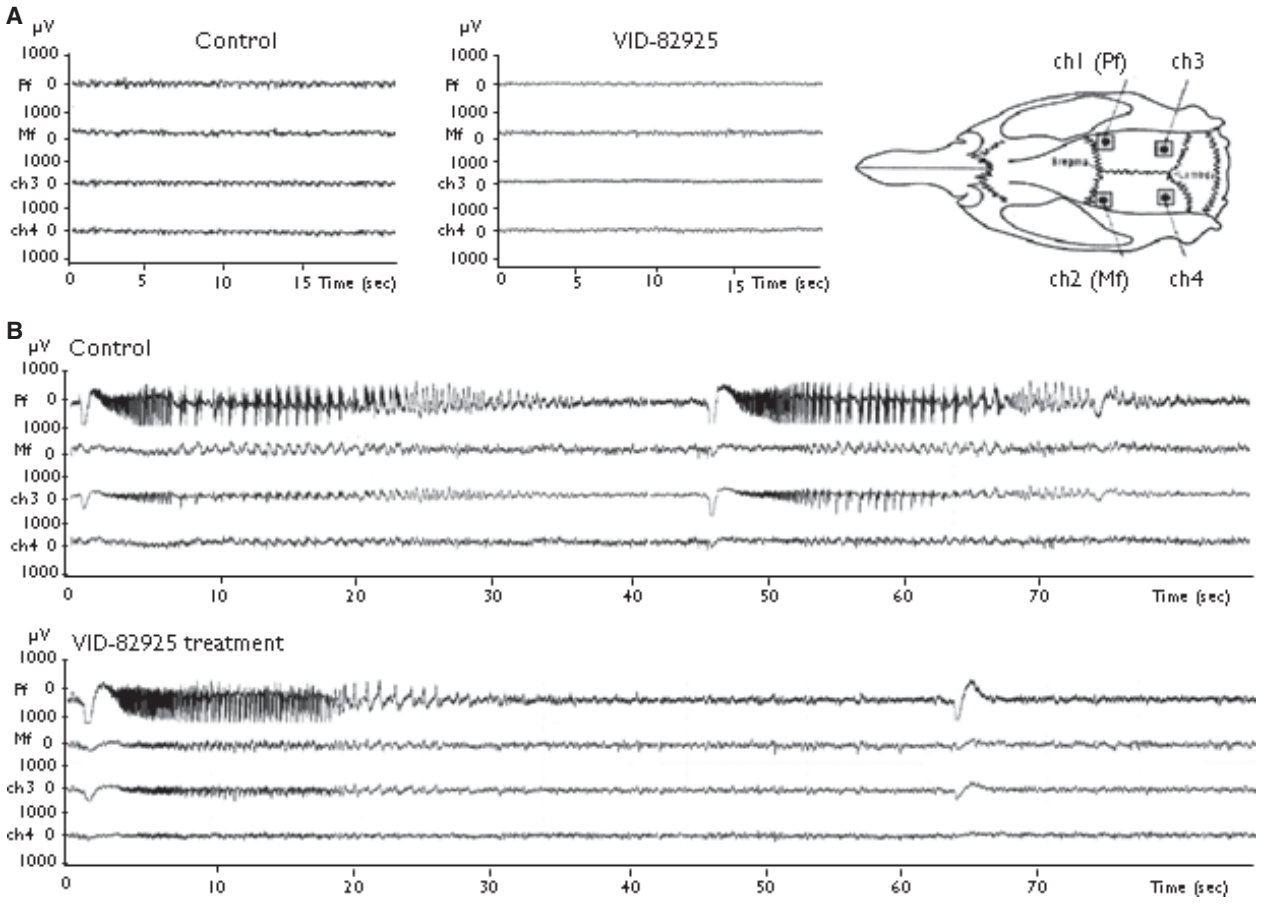

Figure 1.
Representative ECoG samples showing the effects of VID-82925 on the basic cortical electric activity (**A**) and on the manifestation of individual seizures at the already active epileptic focus (**B**). The scheme illustrates the arrangement of the ECoG recording electrodes and the sites of the primary focus (Pf) and mirror focus (Mf).

Table 1. Effects of VID-82925 on the development of the epileptic focus

Time (20 min window)	Number of seizures/20 min			Duration of seizures (s)			Amplitude (mV)		
	Control	VID-82925	Trend	Control	VID-82925	Trend	Control	VID-82925	Trend
20	13 ± 3	9.1 ± 4	↓3.9	16.9 ± 2	15.1 ± 4	↓1.8	0.73 ± 0.1	0.59 ± 0.1	↓0.14
40	12.4 ± 3	6.1 ± 2[a]	↓6.3	35.8 ± 7	29 ± 10	↓6.8	0.85 ± 0.2	0.64 ± 0.1	↓0.21
60	13.2 ± 4	4.9 ± 2[a]	↓8.3	36.6 ± 11	33.8 ± 11	↓2.8	0.86 ± 0.2	0.69 ± 0.1	↓0.17
80	12.8 ± 3	5.3 ± 1[a]	↓7.5	35.1 ± 9	31.4 ± 6	↓3.7	0.95 ± 0.3	0.67 ± 0.1	↓0.28
100	14.8 ± 4	5 ± 1[a]	↓9.8	34 ± 10	33 ± 9	↓1	0.92 ± 0.3	0.71 ± 0.1	↓0.21
120	14.2 ± 4	3.5 ± 1[a]	↓10.7	34.5 ± 11	39 ± 12	↑4.5	0.91 ± 0.4	0.74 ± 0.1	↓0.17
140	15 ± 5	3.8 ± 1[a]	↓11.2	34.5 ± 9	39 ± 14	↑4.5	0.96 ± 0.2	0.75 ± 0.1	↓0.21
160	15.5 ± 5	4 ± 1[a]	↓11.5	34 ± 12	40 ± 15	↑9.4	0.95 ± 0.2	0.76 ± 0.1	↓0.19
180	15.7 ± 3	4.4 ± 1[a]	↓11.3	35.6 ± 9	45 ± 8	↑9.4	1 ± 0.2	0.77 ± 0.1	↓0.23
200	15.9 ± 3	8.8 ± 2	↓7.1	35.6 ± 8	47 ± 8[a]	↑11.4	1 ± 0.1	0.82 ± 0.1	↓0.18
220	15.7 ± 3	10 ± 2	↓5.7	35.6 ± 8	48 ± 4[a]	↑12.4	1 ± 0.2	0.83 ± 0.1	↓0.17

The data illustrate the effects of VID-82925 on the number of seizures, on duration of seizures, and on the amplitude of epileptiform discharges in the pretreatment experiments. Data are expressed as mean ± SD. Significance criterion: $p < 0.05$.
[a]Significant changes.

were still only 83% of the control value at the 220th min in pretreated animals.

Depressing effect of VID-82925 on the ictogenesis of the already stable epileptic focus

The effects of VID-82925 on the ictogenesis of the already stable epileptic focus turned out to be even more remarkable than its effects on the development of the focus. The decrease in the number of seizures first became significant at the 40th min after the application of VID-82925 (Table 2). The number of seizures reached its lowest level (28% of control value) at the 180th min; it then increased but was still significantly below the control level at the 260th. The decrease in the duration of seizures first became significant at the 20th min (Table 2). The durations were the shortest (57% of the control value) at the 140th min, and then increased gradually and approached the control level at the 240th min, and at the 260th min became 116% of the control value. The over increase in the durations could be the indication of a rebound (withdrawal) phenomenon after the decline of the effects of VID-82925. It was rather imposing that during the most effective phase of VID-82925, the duration of seizure-free periods was as long as 183.3 ± 10 s in comparison to the control value of 16.1 ± 5 s (Fig. 1B). In some VID-82925–treated animals even 10 min elapsed between the manifestations of succeeding seizures. The decrease in the summated ictal activity first became significant at the 20th min (Fig. 2B). Then summated ictal activity decreased further and reached its minimum at the 140th min (19% of the control value), and stayed at considerably depressed level even until the 240th min, and was still only 67% of the control value at the 260th min.

Despite the remarkable depressed epileptiform activity under the influence of VID-82925, the amplitudes of seizure discharges did not modify noticeably (Fig. 1B and Table 2).

Comparison between the efficiency of VID-82925 and the reference AEDs

In levetiracetam pretreatment experiments, the epileptiform activity did not differ considerably from that of control (Fig. 2A). In contrary, pretreatment with VID-82925 exerts a clear depressing effect, indicated by a significantly reduced epileptiform activity, measured during the developing phase, but even at 180 min (see details earlier).

In treatment experiments the antiepileptic effects of VID-82925 and of the reference drugs on the epileptiform activity of the stable focus were comparable in intensity; however, the effect of VID-82925 was 2–3 times longer. The carbamazepine decreased the summated ictal activity down to one fifth of the control value at its peak effect, and this activity lasted approximately as long as 80 min (Fig. 2B). The levetiracetam decreased the summated ictal activity slightly below one third of the control value, and this activity lasted approximately as long as 100 min. The VID-82925 decreased the summated ictal activity slightly below one fifth of the control value, and its effects lasted more than 180 min (see details earlier).

Possible blocking effect of VID-82925 on the GJal communication

In treatment experiments, when VID-82925 was administered first and after 100 min followed by carbenoxolone, no further depression of the summated ictal activity was observed; neither was the duration of the effect of VID-82925 modified in the presence of carbenoxolone (Fig. 3A). When carbenoxolone was applied first and after 10 min followed by VID-82925, the summated ictal activity was further decreased both in intensity and duration in comparison to that when carbenoxolone was applied alone (Fig. 3B). The degree and the duration of reduction of the summated ictal activity in the presence of both carbenoxolone and VID-82925

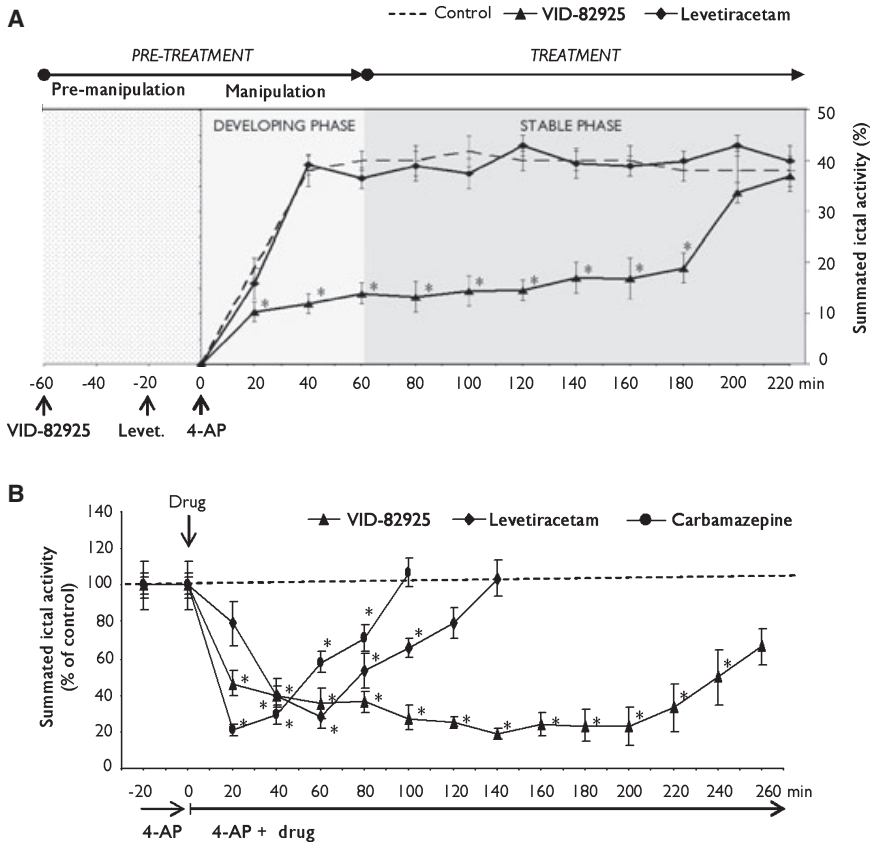

Figure 2.
Effects of pretreatment with VID-82925 (n = 10) or with levetiracetam (n = 5) (**A**) and treatment with VID-82925 (n = 10), or with levetiracetam (n = 5), or with carbamazepine (n = 6) (**B**), respectively, on the seizure activity induced by 4-AP (considered as control). In **A**: Pretreatment means both the premanipulation of the cortex by the tested drug before 4-AP application (dotted panel) and the manipulation of the cortex during the developing phase of the epileptic focus (light shaded panel). The stable phase of the focus is represented by the dark shaded panel. In **B**: Treatment means the application of the drugs during the stable phase of the focus, approximately 60 min after the application of 4-AP (see dark shaded panel in Fig. 2A). The ongoing treatment is indicated below each vertical arrow. −20 on the time scale represents the last 20 min of the preceding 60-min seizure activity induced by 4-AP (considered as control), just before the application of drugs. Data are expressed as mean ± SD. *Significant changes. Significance criterion: $p < 0.05$.

were very similar to that when VID-82925 was administered alone.

Effects of VID-82925 on heart activity

In pretreatment experiments, the VID-82925 slightly decreased the heart frequency, whereas the amplitudes of QRS complex transiently decreased during the 60 min of the pretreatment in comparison to control (Fig. 4A). Despite these changes, both parameters remained in the physiologic intervals and returned to the control level after the induction of epileptiform activity. In treatment experiments, the ECG did not show any considerable changes either in the heart frequency or in the amplitudes of QRS complex in the presence of VID-82925 (Fig. 4B).

Discussion

The aim of the present study was to investigate the effects of the ''VID-82925'' marked kinase inhibitor molecule both on the development of the epileptic focus as well as on the epileptiform activity of the already stable epileptic focus of anaesthetised rats using the 4-AP–induced in vivo epilepsy model. The data of VID-82925 were compared to those of known AEDs and carbenoxolone.

Our results reveal that although the VID-82925 pretreatment does not prevent completely the activation of an epileptic focus, it suppresses significantly the manifestation of the epileptiform activity not only during the developing phase, but the epileptiform activity continues to remain at a

Table 2. Effects of VID-82925 on the epileptiform activity of the already stable epileptic focus

	Time (20 min window)	Number of seizures/20 min	Trend	Duration of seizures (s)	Trend	Amplitude (mV)	Trend
Control		17.1 ± 4		38.3 ± 10		0.85 ± 0.1	
VID-82925	20	14 ± 3	↓3.1	24.6 ± 7	↓13.7	0.79 ± 0.1	↓0.06
VID-82925	40	11.8 ± 2[a]	↓5.3	24.5 ± 7[a]	↓13.8	0.80 ± 0.1	↓0.05
VID-82925	60	11.3 ± 5[a]	↓5.8	23.3 ± 7[a]	↓15	0.79 ± 0.1	↓0.06
VID-82925	80	10.4 ± 4[a]	↓6.7	24 ± 4[a]	↓14.3	0.78 ± 0.1	↓0.07
VID-82925	100	6.8 ± 3[a]	↓10.3	23.3 ± 10[a]	↓15	0.80 ± 0.1	↓0.05
VID-82925	120	6.4 ± 1	↓10.7	24,4 ± 4[a]	↓13.9	0.81 ± 0.1	↓0.04
VID-82925	140	5.3 ± 1[a]	↓11.8	21.9 ± 13	↓16.4	0.81 ± 0.1	↓0.04
VID-82925	160	5.3 ± 1[a]	↓11.8	26.1 ± 4[a]	↓12.2	0.83 ± 0.1	↓0.02
VID-82925	180	4.7 ± 2[a]	↓12.4	26.5 ± 9[a]	↓11.8	0.82 ± 0.2	↓0.03
VID-82925	200	4.9 ± 2[a]	↓12.2	27 ± 7[a]	↓11.3	0.81 ± 0.1	↓0.04
VID-82925	220	5.3 ± 3[a]	↓11.8	28.2 ± 10	↓10.1	0.79 ± 0.1	↓0.06
VID-82925	240	7.4 ± 2[a]	↓9.7	37.8 ± 10	↓0.5	0.86 ± 0.1	↑0.01
VID-82925	260	8.2 ± 4[a]	↓8.9	44.5 ± 10	↑6.2	0.87 ± 0.1	↑0.02

The data illustrate the effects of VID-82925 on the number of seizures, on duration of seizures and on the amplitude of epileptiform discharges in the treatment experiments. Data are expressed as mean ± SD. Significance criterion: $p < 0.05$.
[a]Significant changes.

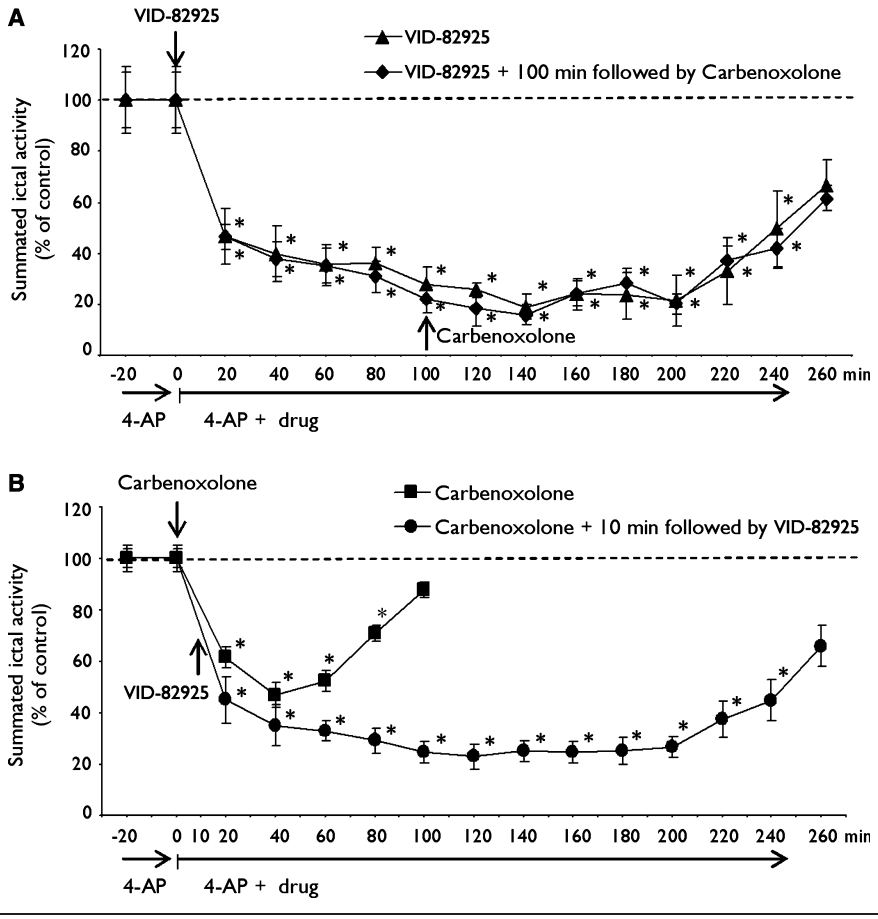

Figure 3.
Effects of treatment with VID-82925 applied alone (n = 10) or together with carbenoxolone (n = 10) (**A**) and treatment with carbenoxolone applied alone (n = 10) or together with VID-82925 (n = 10) (**B**) on the summated ictal activity. In **A**: In the already epileptic animals, the VID-82925 was administered first and after 100 min carbenoxolone was applied. In **B**: In the already epileptic animals, the carbenoxolone was applied first and after 10 min VID-82925 was administered. The ongoing treatment is indicated below each vertical arrow. −20 on the time scale represents the last 20 min of the preceding 60-min seizure activity induced by 4-AP (considered as control), just before the application of drugs. Data are expressed as mean ± SD. *Significant changes. Significance criterion: $p < 0.05$.

significantly depressed level for a considerable long time even during the stable phase. Conversely, epileptiform activity in the levetiracetam pretreated animals did not differ considerably from that of controls both during the developing phase and during the stable phase of the focus. From these observations, we can conclude that VID-82925 does have a suppressing effect on the development of the epileptic focus, whereas levetiracetam does not have this kind of

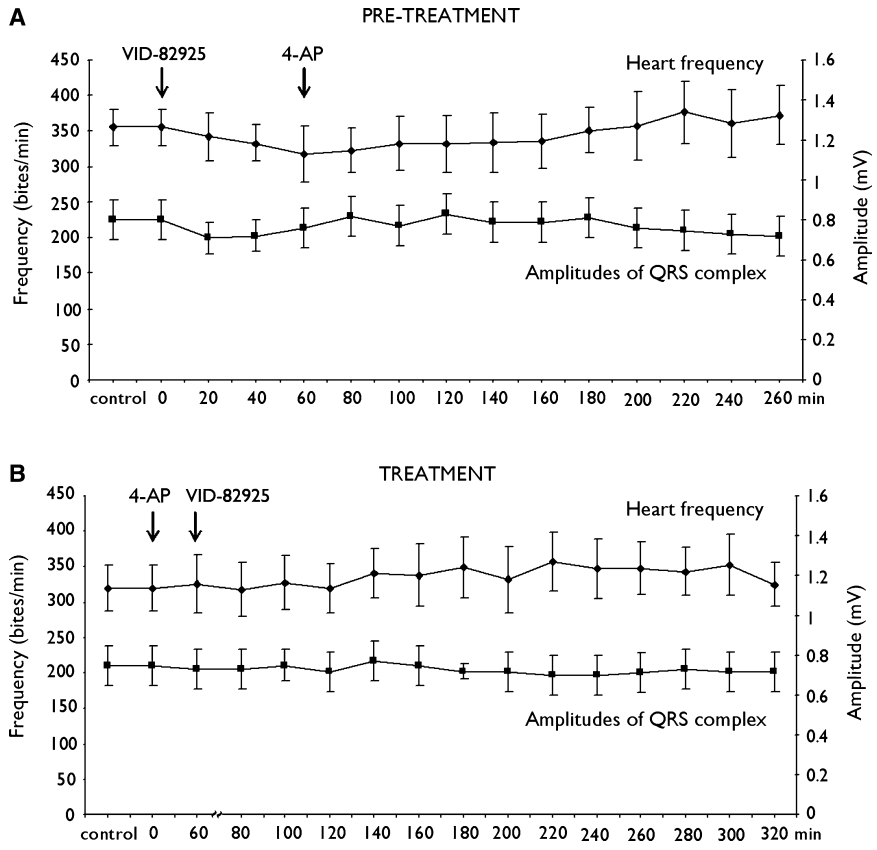

Figure 4.
Effects of pretreatment (**A**) and treatment (**B**) with VID-82925 on the heart frequency and on the amplitudes of QRS complex. Data are expressed as mean ± SD. Significance criterion: $p < 0.05$. *Epilepsia* © ILAE

effect, although in treatment experiments levetiracetam significantly reduced the epileptiform activity of the already stable focus. Although previous experiments in the kindling model suggested that levetiracetam in addition to its seizure-suppressing activity may possess antiepileptogenic activity (Löscher et al., 1998), a recent study by Brandt et al. (2007) in status epilepticus models revealed lack of an antiepileptogenic effect of chronic treatment with levetiracetam. These discrepancies could be explained by the different conditions in the different models.

The long-lasting seizure depressing effect of VID-82925 might be the results of the persistent plastic changes induced by VID-82925 in the cortical tissue early in the process of epileptogenesis (both during the premanipulation of the intact cortex and/or the manipulation of the cortex, during the developing phase of the focus) that alter the subsequent progressive evolution of the epilepsy. However, we cannot exclude a possible treatment effect of the VID-82925 persisting during the stable phase of the focus. These findings further confirm our earlier suggestions that different cellular and network mechanisms could be involved at least partially in the development of the epileptic focus and in the maintenance of seizure activity of an already stable epileptic focus.

The treatment of the animals with VID-82925 during the stable phase of the epileptic focus turns out to be remarkably effective against ictogenesis, indicated by significant reductions in both the numbers and durations of seizures as well as by the considerably longer seizure free periods. The power of the depressing effects of VID-82925 on the epileptiform activity becomes obvious and worth considering if we compare it to the efficacy of the already traded AEDs. The antiepileptic activity of VID-82925 is at least as pronounced as of the tested AEDs; however, the duration of its effect is 2 or 3 times longer. Despite the noteworthy depressing effects of VID-82925 on the epileptiform activity, it apparently does not influence the basic cortical electric activity of the rats and does not exert any serious harmful effect on their heart activity.

Currently there are about 20 different AEDs available on the market and some compounds in various stages of clinical phases (Rogawski, 2006), but none of them are reported to aim at the modulation of the action of protein kinases. These enzymes have major roles in signal transduction pathways with broad substrate specificity, indicating their involvement in many cellular events, both physiologic and pathologic, including epilepsy. It has been shown that increased activity of different kinases can contribute to the dysfunction of both ion (Bernard et al., 2004) and neurotransmitter receptor channels (Tehrani & Barnes, 1995; Rakhade et al., 2008) that are involved in several types of

epilepsy. Inhibition of several kinases has also been reported to reduce seizure activity (Weisner et al., 1999; Inan & Büyükafsar, 2008; Queiroz & Mello, 2008) and to have antiepileptogenic properties in genetic epilepsy (Zeng et al., 2009).

Several reports confirm that phosphorylations can also regulate GJal communication at multiple levels. Protein kinases are involved not only in several points of the lifecycle of the GJ channels [starting from the gene expression of the GJ channel forming proteins (connexin, Cx) through the trafficking of Cxs and their assembly into functioning channels as well as their disassembly and degradation] but also in the gating of GJ channels (Cruciani & Mikalsen, 2002; Laird, 2005; Moreno & Lau, 2007). Phosphorylation is demonstrated to modify the permselectivity of the GJ channels formed by different Cxs to specific signaling molecules (Goldberg et al., 2002). It has been reported that both neuronal and glial Cxs have sites for phosphorylation for a number of protein kinases (Lampe & Lau, 2004). Most of the data available are related to Cx43 (astrocyte specific Cx), showing that activation of protein kinase A, protein kinase C, p34(cdc2)/cyclin B kinase, casein kinase 1, mitogen-activated protein kinase, and pp60(src) kinase can lead to phosphorylation of the majority of the 21 serine and 2 of the tyrosine residues in the C-terminal region of Cx43 (Lampe & Lau, 2004).

Our results indicate that in the presence of VID-82925, the GJ channel blocker carbenoxolone does not exert additional depressing effect on the epileptiform activity. Carbenoxolone is known to reduce effectively the seizure activity in different epilepsy models both in vitro (Jahromi et al., 2002; Gigout et al., 2006) and in vivo (Szente et al., 2002; Gajda et al., 2006; Nilsen et al., 2006; Medina-Ceja et al., 2008). This may be the indication that VID-82925 and carbenoxolone are in competition at least partially on influencing GJal communication. Because VID-82925 is able to exert additional depressing effect on the epileptiform activity in both intensity and duration in the presence of carbenoxolone, it seems possible that VID-82925 has more complex effects on seizure activity than just blocking of GJal communication. The VID-82925 influenced the duration of seizures and the amplitudes of seizure discharges differently depending on the actual phase of the focus. These observations indicate that VID-82925 might affect partially distinct mechanisms during the development of an epileptic focus and during the activity of an already stable focus.

Our results suggest that VID-82925 might provide novel strategy for epilepsy treatment that might be effective not only for the currently drug-resistant epilepsies, but also for suppressing the development of a stable epileptic focus targeting primary cell signaling pathways that trigger the downstream mechanisms underlying epileptogenesis. Because the VID-82925 was administered intravenously, its remarkable antiepileptic effects suggest that the compound crosses the blood–brain barrier successfully, an essential feature of a potential antiepileptic drug. However, more exact molecular biologic investigation must be carried out to identify the entire cellular targets of VID-82925, and detailed pharmacokinetic, pharmacodynamic, and toxicokinetic analysis are needed, but these investigations are beyond of the scope of the present study.

Considering the complex role of various protein kinases in cellular signaling pathways, it seems likely that modulation of their activities might open new possibilities for epilepsy prophylaxis and therapy.

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Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's positions on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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