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D-23129: a potent anticonvulsant in the amygdala kindling model of complex partial seizures

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Abstract

The novel anticonvulsant drug D-23129 (*N*-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester) was evaluated in the amygdala kindling model of complex partial seizures in rats. D-23129 exerts potent anticonvulsant activity against both focal and generalized seizures in animal models of epilepsy. After intraperitoneal and oral administration in kindled rats, the substance dose dependently increased the threshold for induction of afterdischarges, exerting significant effects already after 0.01 mg/kg. In higher doses (2.5–5 mg/kg i.p., 10–15 mg/kg p.o.) D-23129 also exerted anticonvulsant effects on other seizure parameters of amygdala-kindled rats, i.e. seizure severity, seizure duration, total duration of behavioural changes and afterdischarge duration. The adverse effects of D-23129 were quantitated in the open field and in the rotarod test, a standard test for motor impairment. D-23129 exerted no adverse effects on behaviour in doses up to 5 mg/kg i.p. and 15 mg/kg p.o. Comparing the adverse effects between kindled and non-kindled rats, no differences were found. The data demonstrate that D-23129 is more potent in the amygdala kindling model of complex partial seizures than in other seizure models. D-23129 is orally active and is devoid of neurotoxic effects in anticonvulsant doses, thus indicating that this compound has potential for antiepileptic therapy.

Keywords: D-23129; Amygdala kindling; Anti-epileptic drug; Valproic acid; Afterdischarge threshold

1. Introduction

D-23129 (*N*-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester Fig. 1) is a novel potential antiepileptic drug, structurally unrelated to the major drugs in current use.

D-23129 was evaluated using a series of in vivo and in vitro testing procedures. The substance has a broad effectivity and high potency against supramaximally induced seizures in the maximal electroshock seizure (MES) test and s.c. pentylenetetrazole test. The compound is also highly effective in genetic models of epilepsy, i.e. in DBA/2 mice (Rundfeldt et al., 1994; Tober et al., 1994a; Rostock et al., 1996) and in GEPR-3 (genetically epilepsy prone rats) and GEPR-9 rats (Dailey et al., 1995). In an in vitro test system, the rat hippocampal slice with chemically induced hyperexcitability and epileptiform activity, D-23129 reversed the effects of 4-aminopyridine to a greater extent than any tested reference compound (Yonekawa et al., 1995).

First investigations about the mechanism of anticonvulsant action of D-23129 indicate that the compound blocks Na^+ and Ca^{2+} currents and potentiates γ-aminobutyric acid (GABA)-induced currents in neuronal cells (Tober et al., 1994b; Rundfeldt et al., 1995).

Despite the optimal use of available anticonvulsant drugs, 20–30% of people suffering from epilepsy have seizures that are resistant to treatment. Complex partial seizures, the most frequent type of seizures in humans, are the seizure types with the highest percentage of drug resistance. About 70% of these patients fail to experience seizure control (Löscher and Schmidt, 1994).

The closest experimental approximation of these psychomotor epilepsies is the kindling model, in which repeated electric stimulation (with an initially subconvulsive current) of the amygdala, hippocampus, or other regions of the limbic system results in the development of focal seizures strikingly similar to those of complex partial seizures of presumed temporal origin in humans. If the

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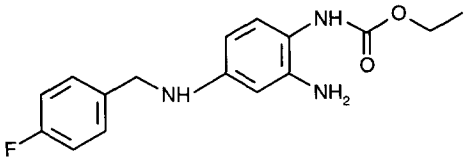

Fig. 1. Chemical structure of D-23129 (*N*-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester).

repeated electrical stimulation is continued, secondarily generalized seizures develop in addition to focal seizures. Once developed, the enhanced sensitivity to electric stimulation seems to be permanent (Goddard et al., 1969; Löscher and Schmidt, 1994; McNamara et al., 1993; Moia et al., 1994). The kindling model is now one of the most extensively used animal models of partial epilepsy, both with respect to studies on the mechanisms of generation and propagation of seizures and for the development of anticonvulsant drugs against partial epilepsy (Stark et al., 1990; Wada, 1986; Löscher and Schmidt, 1988). The kindling model is a model of chronic brain dysfunction and leads not only to enhanced seizure susceptibility but also to chronic disturbances of behaviour and altered susceptibility to psychotropic adverse effects of drugs (Löscher and Hönack, 1991; Löscher and Schmidt, 1993).

The aim of the present study was to investigate the effectiveness of D-23129 in the amygdala kindling model in rats. In all experiments, behavioural alterations of the rats in response to D-23129 were closely monitored. To exclude effects on the protective index induced by the kindling procedure, the effective dose inducing neurotoxicity in kindled and non-kindled rats was compared.

Some of the data have been published in abstract form (Tober et al., 1994a,b; Rundfeldt et al., 1994).

2. Materials and methods

2.1. Animals

Female Wistar rats (Charles River, Sulzfeld) weighing 220–260 g were used. The animals were purchased from the breeder at a body weight of 130–200 g. All animals were allowed to become acclimated for at least two weeks before being used in the experiments. The rats were conventionally kept in groups of five in plastic cages. Standard laboratory chow (ssniff MIR 15, Spezialdiäten, Soest/Westfalen) and tap water were allowed ad libitum. All experiments were done in a laboratory under controlled environmental conditions (ambient temperature 23–25°C, humidity 50–60%, 12/12 h light/dark cycle, light on at 6:00 a.m.).

2.2. Preparation of animals

The rats were anaesthetised with chloral hydrate (360 mg/kg i.p.) and received stereotaxic implantation (according to the surgery methods described in the atlas of Paxinos and Watson (1986)) of one bipolar electrode in the right basolateral amygdala. Coordinates for electrode implantation were AP −2.2, L −4.7, V −8.7. All coordinates were measured from bregma. The electrode consisted of two twisted teflon-coated 0.2 mm diameter stainless steel wires separated by 0.5 mm at the tip. The size, shape and resistance of the bipolar electrode were the same for all kindled rats used for the present study. A skull screw, positioned over the contralateral parietal cortex, served as the indifferent reference electrode. Bipolar and reference electrodes were connected to plugs and the electrode assembly and anchor screws were held in place with dental acrylic cement applied to the exposed skull surface. After implantation of electrodes the rats were housed in individual cages.

2.3. Kindling

Using an electrical stimulator (Stimulator I, Hugo Sachs Elektronik, March) electrical stimulation of the amygdala was initiated after a recovery period of at least 2 weeks after surgery.

Constant current stimulations (500 μA, 1 ms, monophasic square-wave pulses, 50/s for 1 s) were delivered to the amygdala once daily (except weekends) until ten consecutive stage 5 seizures were elicited.

2.3.1. Seizure severity

Seizure severity was classified according to Racine (1972):

1. immobility, eye closure, twitching of vibrissae, sniffing, facial clonus;
2. head nodding associated with more severe facial clonus;
3. clonus of one forelimb;
4. rearing, often accompanied by bilateral forelimb clonus;
5. rearing with loss of balance and falling accompanied by generalized clonic seizures.

2.3.2. Afterdischarge threshold

Electrical activity of the amygdala was recorded from the bipolar electrode on a chart recorder (Linearcorder WR 3310, Hugo Sachs Elektronik, March) before and after stimulus delivery.

The electrical susceptibility of the stimulated region for triggering paroxysmal neuronal activity (threshold for induction of afterdischarges) was recorded on the first stimulation after electrode implantation as well as after kindling acquisition, using an ascending stairstep procedure (Freeman and Jarvis, 1981). The initial current intensity was 10 μA, and the current intensity was increased in steps of about 20% of the previous current at intervals of 1 min until an afterdischarge of at least 3 s duration was elicited.

Afterdischarges, a measure of electrographic seizure

activity from the amygdala, were the EEG spikes with an amplitude of at least two times the amplitude of the prestimulus recording and a frequency greater than 1/s.

Determination of the threshold for induction of afterdischarges was repeated at intervals of 2–3 days until all animals exhibited reproducible seizure thresholds. Since almost all fully kindled animals exhibited generalized seizures (stage 4–5) at the afterdischarge threshold current, it was not necessary to determine the threshold for generalized seizures separately.

In addition to the threshold for induction of afterdischarges, in fully kindled rats the following parameters of kindled seizures were measured during stimulation with the afterdischarge threshold current:

Seizure severity was classified as described above.

2.3.3. Seizure duration

Seizure duration was the duration of limbic (stages 1–2) and/or motor seizures (stage 3–5); limbic seizure activity (immobility associated with low-amplitude afterdischarges and occasional facial clonus or head nodding) which often occurred after termination of motor seizures was not included in seizure duration.

2.3.4. Duration of behavioural changes

Total duration of behavioural changes was the duration of limbic and/or motor seizures (seizure duration) and the period between the end of the seizures and the onset of normal locomotion with sometimes immobility, stereotyped behaviour (wet dog shakes, head nodding), occasional facial clonus and a few single EEG spikes.

2.3.5. Afterdischarge duration

Afterdischarge duration was the total duration of afterdischarges.

2.4. Evaluation of drug effects on focal seizure threshold

The effect of D-23129 was assessed in groups of 9–14 fully kindled rats by determination of the threshold for induction of afterdischarges, i.e. the most sensitive measure of anticonvulsant effects on focal seizure activity in kindled rats. In addition to the threshold for induction of afterdischarges, the influence of D-23129 on seizure severity, seizure duration, total duration of behavioural changes and duration of afterdischarges was also measured during stimulation with the afterdischarge threshold current.

Pretreatment times used for drug testing were chosen on the basis of previous time course experiments with the drug in the maximal electroshock seizure test in rats (Rostock et al., 1996).

The control threshold for induction of afterdischarges was determined 2 days prior to each drug treatment.

After establishment of stable control thresholds, stimulation was initiated three 20% steps below the previously determined individual control threshold 10 min and 60 min after i.p. and p.o. application of drug or vehicle, respectively. For control determinations, rats received i.p. or p.o. application of the vehicle (0.5% hydroxyethyl cellulose in water) with the same pretreatment time used for drug testing. At least 7 days were interposed between two drug applications in order to prevent or minimise changes in drug effects due to long-lasting alterations in receptor function or due to drug cumulation or tolerance.

The anticonvulsant activity of valproate was evaluated in additional experiments with another group of 12 fully kindled rats. Valproate was investigated with a pretreatment time of 15 min. Experiments were done as described above.

2.5. Drugs

D-23129 was synthesised at ASTA Medica (Frankfurt/Main) and valproate (as sodium salt) was obtained from Synopharm (Barsbüttel). Both substances were suspended, in a mortar, in 0.5% hydroxyethyl cellulose in water and administered in a volume of 0.5 ml/100 g body weight in rats. D-23129 was injected i.p. in doses of 0.01–5 mg/kg and p.o. in doses of 0.01–15 mg/kg. Valproate was investigated i.p. in doses of 10–200 mg/kg.

2.6. Evaluation of behavioural effects

Behavioural alterations after administration of the test drug were determined at different times after administration of the compound up to 3 min prior to amygdala stimulation. Animals were taken out of the home cage, placed in an open field (a black box of ellipsoidal shape with black walls, 70–100 cm diameter) and observed for about 1 min. Ataxia, hyperlocomotion, head weaving, stereotyped sniffing, biting, licking or grooming, reciprocal forepaw treading ('piano playing'), stereotyped rearing, hyperexcitability (as indicated by increased reactions to noise or handling), tremor, abduction of hind limbs, reduction of righting reflexes, flat body posture, circling, straub tail and piloerection were scored using a ranked intensity scale where 0 = absent, 1 = equivocal, 2 = present and 3 = intense.

Behavioural alterations other than those described above were recorded separately. In addition to rating of motor impairment in the open field, impaired motor function was quantitated by the rotarod test (diameter of the rod 7 cm, 8 rotations/min). Neurological deficit was indicated by the inability of the animals to maintain their equilibrium for at least 1 min on the rotating rod. The kindled rats were trained prior to drug experiments to remain on the rod. Untreated but trained rats were able to remain on the rod for several minutes. After drug treatment, rats which were not able to maintain their equilibrium on the rod for 1 min were allowed two more trials. Only animals that were not able to remain on the rod for 1 min were considered to exhibit neurological deficit.

Rectal body temperature was measured with an electronic thermometer (ama-digit ad 30 th, Amarell Electronic, Kreuzwertheim) before substance administration and 3–2 min before electrical stimulation.

The weight of the animals was recorded daily before drug injection.

To rule out changes in protective index due to epileptogenesis in the kindled rats, the neurotoxic effects of D-23129 on kindled and non-kindled rats were compared in a separate study with the kindled rats used for the experiments described above and 10 age-matched non-kindled rats. Four doses of D-23129 (5, 7, 10 and 20 mg/kg) were injected i.p. and motor impairment was determined on the rotarod 10 min after drug application. From these data, the TD_{50} for both groups was calculated.

2.7. Histology

After termination of the experiments, placement of stimulating electrodes was examined histologically in all animals. The rats were anaesthetised with pentobarbital sodium (50 mg/kg i.p.) and perfused transcardially with a fixative consisting phosphate-buffered formaldehyde (0.2 M, pH 7.4). 2 h later, the brains were removed and postfixed several days. Serial sections (50 μm) of the part of the brain with the right basolateral amygdala were cut coronally with a vibratome (vibroslice, TSE, Kronberg). The sections were mounted on glass slides and stained with toluidine blue (0.1%). Only animals with electrodes in the right basolateral amygdala were used for further evaluation.

2.8. Statistics

All data are given as means ± S.E.M. Significance of differences between seizure parameters before and after substance administration in the same group of rats was calculated by the Wilcoxon signed-rank test for paired replicates. The significance of differences of behavioural alterations was evaluated by the U-test of Mann and

Table 1
Effect of intraperitoneal and oral administration of D-23129 on the threshold for induction of afterdischarges, seizure severity, seizure duration, total duration of behavioural changes and afterdischarge duration of fully kindled rats at stimulation with the afterdischarge threshold current

Treatment	Dose (mg/kg)	Administration	Threshold for induction of afterdischarges (μA)	Seizure recordings at afterdischarge threshold current			
				Seizure severity (score)	Seizure duration (s)	Total duration of behavioural changes (s)	Afterdischarge duration (s)
Control	0	i.p.	46.1 ± 5.4	4.9 ± 0.1	38.6 ± 2.3	232.5 ± 13.6	78.8 ± 8.7
D-23129	0.01	i.p.	54.9 ± 6.2 [a]	4.5 ± 0.3	37.7 ± 7.2	228.2 ± 36.5	84.1 ± 18.5
Control	0	i.p.	53.6 ± 5.9	4.5 ± 0.3	36.2 ± 6.1	194.5 ± 34.6	71.4 ± 14.1
D-23129	0.1	i.p.	60.2 ± 7.1 [a]	4.6 ± 0.3	34.1 ± 3.7	239.5 ± 34.0	86.3 ± 14.1
Control	0	i.p.	59.3 ± 7.8	4.8 ± 0.1	42.6 ± 4.8	211.8 ± 19.5	83.3 ± 10.1
D-23129	1.0	i.p.	104.2 ± 10.3 [c]	4.5 ± 0.2	29.3 ± 3.3 [b]	221.8 ± 28.0	75.3 ± 9.7
Control	0	i.p.	69.9 ± 9.1	4.8 ± 0.1	39.9 ± 4.0	231.3 ± 23.2	84.8 ± 16.4
D-23129	2.5	i.p.	126.3 ± 22.3 [b]	4.2 ± 0.2 [b]	31.2 ± 3.8	224.8 ± 37.4	62.2 ± 10.1
Control	0	i.p.	85.7 ± 13.9	4.9 ± 0.1	38.8 ± 3.2	232.1 ± 16.0	84.9 ± 11.0
D-23129	5.0	i.p.	373.3 ± 103.5 [b]	3.8 ± 0.3 [b]	7.7 ± 1.0 [b]	22.0 ± 3.3 [b]	7.0 ± 0.9 [b]
Control	0	p.o.	38.1 ± 6.0	4.9 ± 0.1	53.7 ± 4.3	253.8 ± 23.9	101.7 ± 16.1
D-23129	0.01	p.o.	56.6 ± 9.7 [b]	4.7 ± 0.3	56.7 ± 5.2	247.2 ± 24.7	94.6 ± 5.6
Control	0	p.o.	41.4 ± 5.7	4.9 ± 0.1	49.8 ± 1.8	276.4 ± 18.8	111.4 ± 24.4
D-23129	0.1	p.o.	51.0 ± 8.1 [a]	5.0 ± 0	44.9 ± 2.9	293.2 ± 14.7	88.1 ± 6.3
Control	0	p.o.	44.7 ± 6.0	4.9 ± 0.1	47.1 ± 1.8	248.3 ± 13.1	75.3 ± 6.5
D-23129	1.0	p.o.	72.2 ± 9.8 [b]	5.0 ± 0	44.9 ± 6.0	251.2 ± 30.6	76.4 ± 10.1
Control	0	p.o.	53.8 ± 8.4	4.7 ± 0.2	44.5 ± 4.1	229.7 ± 17.3	70.1 ± 7.8
D-23129	5.0	p.o.	125.5 ± 14.0 [b]	4.9 ± 0.1	43.2 ± 4.2	213.1 ± 24.3	113.3 ± 35.8
Control	0	p.o.	44.9 ± 5.8	4.5 ± 0.3	45.5 ± 3.8	204.6 ± 19.2	76.6 ± 8.5
D-23129	10.0	p.o.	174.2 ± 26.1 [b]	4.2 ± 0.2	25.9 ± 3.4 [b]	101.6 ± 22.0 [b]	41.9 ± 10.1 [b]
Control	0	p.o.	50.6 ± 7.0	4.4 ± 0.3	43.9 ± 5.0	185.9 ± 25.1	75.8 ± 13.0
D-23129	15.0	p.o.	282.1 ± 65.0 [c]	3.1 ± 0.3 [b]	13.9 ± 3.5 [c]	41.6 ± 18.8 [b]	17.5 ± 7.5 [b]

The test was carried out 10 min after i.p. drug application and 60 min after oral application. The control threshold for induction of afterdischarges was determined 2 days prior to each drug treatment in the same group of animals by using the same vehicle. All data are shown as means ± S.E.M. 9–14 animals per group. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ significantly different from solvent control, Wilcoxon signed-rank test for paired replicates.

Whitney. The TD_{50} for motor impairment was calculated by probit regression.

3. Results

3.1. Effect of D-23129 in the amygdala kindling model

Based on the previously determined time of peak effect in the maximal electroshock seizure test (Rostock et al., 1996), D-23129 was tested in the amygdala kindling model in rats with pretreatment times of 10 min and 60 min after intraperitoneal and oral administration, respectively. D-23129 exhibited potent and dose-dependent anticonvulsant effects in amygdala-kindled rats after intraperitoneal and oral administration (Table 1). Thus, a significant increase in afterdischarge threshold was already observed with the small doses of 0.01 and 0.1 mg/kg after both intraperitoneal and oral administration (Fig. 2).

The effect on afterdischarge threshold increased with higher doses of D-23129. At 1 mg/kg i.p. and p.o., D-23129 elevated the afterdischarge threshold by 93% and 72%, and at 5 mg/kg by 342% and 202%, respectively.

After intraperitoneal administration, D-23129 also reduced the seizure duration at 1 mg/kg and the seizure severity at 2.5 mg/kg after stimulation with the afterdischarge threshold current. Increasing the dosage to 5 mg/kg i.p. resulted in a significant reduction of all seizure parameters recorded at the elevated afterdischarge threshold current, i.e. seizure severity, seizure duration, total duration of behavioural changes and afterdischarge duration.

After oral administration, the seizure parameters other than the threshold were influenced by higher doses of D-23129. Only at 10 mg/kg p.o., did D-23129 significantly decrease seizure duration, total duration of behavioural changes and afterdischarge duration. Increasing the dosage to 15 mg/kg p.o. resulted in a significant reduction of all measured seizure parameters, i.e. seizure severity, seizure duration, total duration of behavioural changes and afterdischarge duration.

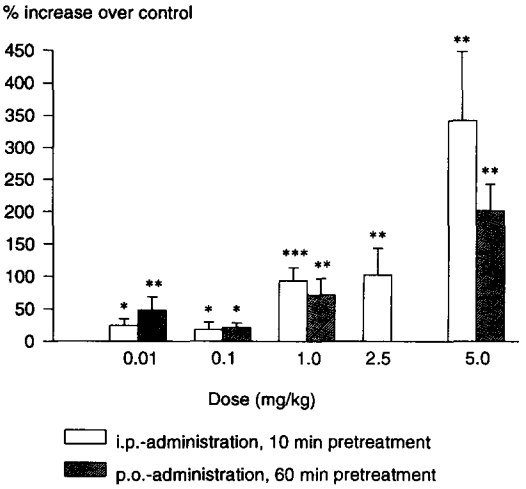

Fig. 2. Effects of D-23129 on the threshold for induction of afterdischarges (ADT) in comparison with vehicle control (= 0%) after intraperitoneal and oral administration. Control experiments were carried out 2 days prior to drug testing in the same group of rats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ threshold for induction of afterdischarges is significantly different from threshold for induction of afterdischarges in control experiments, Wilcoxon signed-rank test for paired replicates.

3.2. Adverse effects of D-23129

Behavioural alterations in response to D-23129 treatment were recorded before amygdala stimulation (7 min

Table 2
Effect of intraperitoneal administration of valproate on the threshold for induction of afterdischarges, seizure severity, seizure duration, total duration of behavioural changes and afterdischarge duration of fully kindled rats at stimulation with the afterdischarge threshold current

Treatment	Dose (mg/kg)	Administration	Threshold for induction of afterdischarges (μA)	Seizure recordings at afterdischarge threshold current			
				Seizure severity (score)	Seizure duration (s)	Total duration of behavioural changes (s)	Afterdischarge duration (s)
Control	0	i.p.	63.6 ± 12.3	5.0 ± 0	37.7 ± 3.5	257.0 ± 9.3	61.7 ± 7.2
Valproate	10	i.p.	69.4 ± 13.4	4.7 ± 0.3	40.1 ± 4.3	216.3 ± 22.2	69.7 ± 8.1
Control	0	i.p.	61.8 ± 12.5	4.9 ± 0.1	46.3 ± 5.1	211.8 ± 14.2	70.4 ± 4.4
Valproate	30	i.p.	83.3 ± 15.6 [b]	4.8 ± 0.2	37.7 ± 4.2	225.5 ± 19.4	77.5 ± 7.7
Control	0	i.p.	67.8 ± 14.6	4.9 ± 0.1	40.5 ± 4.8	195.8 ± 22.6	54.5 ± 6.5
Valproate	50	i.p.	82.3 ± 12.3 [a]	4.9 ± 0.2	38.9 ± 6.9	192.8 ± 32.0	65.3 ± 11.5
Control	0	i.p.	80.3 ± 15.9	4.9 ± 0.1	39.4 ± 4.4	180.4 ± 21.8	49.5 ± 6.3
Valproate	100	i.p.	115.9 ± 19.5 [b]	3.9 ± 0.3	37.8 ± 6.6	157.3 ± 30.6	49.7 ± 9.4
Control	0	i.p.	88.1 ± 36.6	4.7 ± 0.3	32.2 ± 4.4	214.2 ± 29.5	66.4 ± 13.4
Valproate	200	i.p.	346.7 ± 70.1 [c]	2.3 ± 0.2 [c]	21.6 ± 4.3	36.6 ± 10.5 [b]	21.1 ± 5.0b

The test was carried out 15 min after i.p. drug application. The control threshold for induction of afterdischarges was determined 2 days prior to each drug treatment in the same group of animals by using the same vehicle. All data are shown as means ± S.E.M. 12 animals per group. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ significantly different from solvent control, Wilcoxon signed-rank test for paired replicates.

after intraperitoneal drug or vehicle administration and 57 min after oral drug or vehicle administration). The rats were observed for adverse effects in an open field, for their inability to maintain their equilibrium on the rotating rod and for changes in rectal body temperature. No overt behavioural adverse effects in the open field and on the rotating rod were observed in kindled rats after intraperitoneal or oral administration of D-23129 in dose ranges of 0.01 mg/kg i.p. to 5 mg/kg i.p. and 0.01 mg/kg p.o. to 15 mg/kg p.o., respectively. The changes in rectal body temperature before treatment and after treatment did not differ from the changes determined in control trials.

Comparison of the doses needed to elicit motor impairment in the rotarod between kindled and non-kindled rats 10 min after i.p. administration yielded TD_{50} values, calculated from a dose-response curve, of 11.7 (8.4–16.2) mg/kg for kindled and 10.0 (2.9–34.0) mg/kg for non-kindled rats, which were not significantly different.

3.3. Effect of valproate in the amygdala kindling model

In comparison to D-23129, valproate was much less potent in kindled rats (Table 2). Thus, in order to obtain a significant increase of the threshold for induction of afterdischarges, doses of at least 30 mg/kg (180 μmol/kg) i.p. had to be injected, whereas comparative effects of D-23129 were already obtained at 0.01 mg/kg (0.03 μmol/kg) i.p.

Significant effects with valproate on seizure severity were measured only at 200 mg/kg (1200 μmol/kg) i.p. For D-23129 significant effects were seen after 2.5 mg/kg (8 μmol/kg) i.p..

3.4. Adverse effects of valproate

Whereas D-23129 did not cause motor impairment or other adverse effects in kindled rats at the doses needed to influence all seizure parameters (5 mg/kg i.p., 15 mg/kg p.o.), valproate induced ataxia (mean score 1.9 ± 0.1, $P < 0.001$) and motor impairment in the rotarod test (57.1% of the animals were not able to maintain their equilibrium on the rod for 1 min) at this dose (200 mg/kg). Some ataxia (mean score 0.6 ± 0.3) was already observed at 100 mg/kg i.p. valproate.

4. Discussion

D-23129 is a derivative of flupirtine, which is marketed as a centrally acting analgesic. Flupirtine was shown to have anticonvulsant effects in animal models of epilepsy with electrically and chemically induced seizures. In the first clinical trials, flupirtine was clinically effective in the treatment of drug-resistant patients with epilepsy (Seamann et al., 1986). Based on these data the quantitative structure-activity relationship of related compounds was examined (Seydel et al., 1994) and D-23129 and its hydrochloride, D-20444, were finally selected for development.

The broad effectiveness of D-20443 was shown previously (Nickel et al., 1993a,b). For chemical and technological reasons the free base D-23129 was preferred for further development.

In amygdala-kindled rats, i.e. a model of complex partial seizures with secondary generalisation (Löscher and Schmidt, 1988), D-23129 exerted potent anticonvulsant effects against both focal and secondary generalised seizures after induction with afterdischarge threshold current.

D-23129 (0.01–15 mg/kg) very potently increased the threshold for induction of afterdischarges after intraperitoneal and oral administration. Together with data obtained from the threshold determination in another model, it can be concluded that D-23129 is capable of totally suppressing focal seizure activity. Only if the stimulus intensity is raised above the new threshold, can seizures be elicited. Furthermore, D-23129 also markedly reduced the severity and duration of the seizures elicited at the raised threshold, albeit only at higher doses (1–5 mg/kg i.p., 10–15 mg/kg p.o.). In this regard, D-23129 is different from carbamazepine and phenytoin. Both drugs are capable of elevating the threshold without showing effects on other seizure parameters at nontoxic doses (Löscher et al., 1993; Rundfeldt et al., 1990).

No adverse effects were measured in amygdala-kindled rats in doses up to 5 mg/kg i.p. and 15 mg/kg p.o. The results demonstrate the anticonvulsant activity of D-23129 in dosages far below those causing the first signs of side effects in the open field and in the rotarod test. Furthermore, comparison of the dose that elicited motor impairment (TD_{50}) in kindled and non-kindled rats showed there to be no difference between both groups at the time of anticonvulsant testing.

With respect to the effect on focal seizures, the potency of D-23129 was higher than that of valproate, an antiepileptic drug of first choice for clinical use in treatment of epilepsy. Valproate increased the threshold for induction of afterdischarges of comparable intensity in a dose which was 300 times higher (180 μmol/kg vs. 0,03 μmol/kg) than the dose of D-23129. Furthermore, the dose of valproate needed to decrease seizure severity and duration also caused ataxia and motor impairment in the rotarod test. Data comparable to our results with valproate have been described by Löscher et al. (1986). In this study, valproate decreased the seizure severity and seizure duration in amygdala-kindled rats only after 200 mg/kg i.p.

The very high potency of D-23129 in another seizure threshold test was shown previously (Rostock et al., 1996). D-23129 increased the threshold for induction of tonic hind limb extension in the maximal electroshock seizure test in mice and rats by 50% (TID_{50}) after application of 1.6 mg/kg i.p. and 0.7 mg/kg i.p., respectively. Taking this dose and the TD_{50} obtained from the rotarod test, we

calculated the therapeutic index of D-23129 to be equal or better than that of standard anticonvulsants on the market (Rostock et al., 1996). The doses needed to significantly increase the seizure threshold in amygdala-kindled rats still remain substantially lower than the doses needed to increase the threshold in the MES test. This is in contrast to the standard anticonvulsants carbamazepine, phenytoin and valproate, which need higher doses to be effective in the kindling test than in the MES threshold test (Rostock et al., 1996; Rundfeldt et al., 1990; Löscher et al., 1993).

In conclusion, the present data demonstrate that D-23129 is more potent in the amygdala kindling model of complex partial seizures than in other seizure models such as the maximal electroshock seizure test, suggesting that D-23129 is particularly effective against partial seizures. D-23129 is orally active and is devoid of neurotoxic effects in anticonvulsive doses, thus indicating that this compound has potential for antiepileptic therapy.

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