Increased Sensitivity to the Anticonvulsant Effect of Valproate in Aging BN/BiRij Rats

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The aim of the present investigations was to study the influence of increasing age on the pharmacodynamics of valproate in BN/BiRij rats, applying a threshold for electrically induced localized seizure activity as a measure of the anticonvulsant effect. Seven groups of healthy male BN/BiRij rats were used, aged 3, 6, 12, 19, 25, 31, and 37 months. Individual plasma concentration versus anticonvulsant effect relationships were determined during a continuous intravenous infusion of sodium valproate at a rate of 5.5 mg/min/kg. The infusion was terminated when the anticonvulsant effect intensity had reached the maximum attainable level or at a total infusion time of three hours. A nonlinear relationship between valproate concentration and anticonvulsant effect intensity was observed with no maximal effect in the concentration range up to 1200 mg · L^{-1}. With increasing age a parallel shift in the concentration versus anticonvulsant effect relationships toward lower concentrations occurred. Thus increasing age appears to be associated with an increased sensitivity to the anticonvulsant effect of valproate.

KEY WORDS: pharmacodynamics; valproate; aging; anticonvulsive.

INTRODUCTION

Valproate is a broad-spectrum antiepileptic drug, which is used in patients of all age groups (1). Clinical evidence indicates that, in contrast to many other drugs, the incidence of adverse drug reactions is lower in the elderly compared to the very young (2–4). An explanation for this observation is not available yet. Studies on the pharmacokinetics of valproate have documented age-related changes (1,5–8). However, very little is known about age-related changes of its pharmacodynamics. The pharmacological effects of valproate are mediated, at least in part, via GABAergic inhibition (9). In a study on the effect of valproate in the GABA-mediated effects on the hypothalamus–pituitary system, a decreased responsiveness was observed with increasing age (10). Thus apparently, increasing age is associated with a decreased pharmacodynamic sensitivity to at least some effects of valproate. The sensitivity to the anticonvulsant effect of valproate in the elderly remained unknown.

Recent techniques to study the pharmacodynamics of anticonvulsants in small laboratory animals (11) allow the determination of concentration versus anticonvulsant effect relationships in individual rats. This approach has been applied successfully to monitor the anticonvulsant effect of several anti-epileptic drugs including valproate (A. Hoogerkamp *et al.*, unpublished). It therefore provides a suitable basis to quantitate pharmacodynamic changes in the sensitivity to these drugs.

The aim of the present study was to investigate the influence of aging on the concentration versus anticonvulsant effect relationship of valproate in BN/BiRij rats.

MATERIALS AND METHODS

Animals

Seven groups of male BN/BiRij rats (TNO Institute for Ageing and Vascular Research, Leiden, The Netherlands) of different ages (3, 6, 12, 19, 25, 31, and 37 months) were used for the main experiment. The 10, 50, and 90% survival ages of the male BN/BiRij rats are 38.1, 31.7, and 22.8 months, respectively. During the period in which the experiments were performed, the rats were kept solitary in Makrolon cages and in a normal 12-hr light-dark cycle (light between 7:00 AM and 7:00 PM). The temperature was maintained at 22–23°C. They were allowed free access to water (acidified, pH 3–4) and food (Standard diet for Rat, Mouse and Hamster, AM 1410, Hope Farms, Woerden, The Netherlands).

Chemicals

Sodium valproate for intravenous injection was kindly donated by Labaz-Sanofit (Maassluis, The Netherlands).

Animal Experiments

Clinical Biochemical/Pathological Evaluation

Blood concentrations of urea nitrogen, aspartate aminotransferase, alanine aminotransferase, glucose, total plasma protein and albumin concentration, 24-hr urine production, urine osmolarity, and creatinine clearance were determined as described before (12).

Further, an autopsy was performed after the pharmacokinetic/pharmacodynamic investigation. After sacrificing the rats, the following organs were removed: heart, lungs, kidneys, liver, and brain. In addition, all other visible abnormalities were collected. These tissues were examined histologically by a pathologist who was blinded with respect to the outcome of the pharmacokinetic and pharmacodynamic investigations.

Pharmacokinetic/Pharmacodynamic Evaluation

Anticonvulsant effect intensity was determined with a modified classical electroshock test (11). Briefly this technique involves the induction of mild seizure activity by

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the application of bipolar electrical pulse trains (50 pulses · sec^{-1}; total pulse duration, 2 msec) directly to the cortex through permanently implanted electrodes. In this way the threshold for localized seizure activity (TLS) can be determined, which is defined as the minimal current intensity necessary to induce clonic movements of the forelimbs. Under the influence of antiepileptic drugs, a concentration dependent increase in the threshold occurs (A. Hoogerkamp *et al.*, unpublished), which shows that the value of the TLS can, in principle, serve as a measure of anticonvulsant effect intensity. An important feature of this newly developed technique is that, due to the benign nature of the seizure activity, the seizure threshold can be determined repeatedly within the same rat, without causing serious distress. As a result, full concentration anticonvulsant response relationships can be determined in individual rats (13). Both in the absence and in the presence of antiepileptic drugs, the occurrence of a forelimb clonus at the TLS is associated with epileptiform activity in the electroencephalogram (14). Further, several antiepileptic drugs (phenobarbital, phenytoin, carbamazepine, valproate, and ethosuximide) cause an increase in the seizure threshold in the model (A. Hoogerkamp et al., unpublished).

Sodium valproate was administered by continuous intravenous infusion at a rate of 5.5 mg/min · kg through a permanently implanted cannula in the jugular vein. The seizure threshold was determined every 3 min and 14 blood samples of 100 μL were taken from an incision in the tail. The infusion was continued for a maximum of 3 hr. If the threshold reached the maximum attainable level of the instrument earlier, the infusion was stopped at that moment. At the end of the infusion the cerebrospinal fluid, the brain, and the blood were collected, for determination of the distribution of valproate. The anticonvulsant effect intensity was expressed as the elevation of the seizure threshold over the baseline.

Drug Analysis

Valproate concentrations in plasma, plasma ultrafiltrate, cerebrospinal fluid, and brain tissue were determined with a gas chromatographic (GC) method after extraction. To 50 μL plasma or plasma ultrafiltrate or 2.5 to 55 μL cerebrospinal fluid, 100 μL 1 *N* HCl was added and 0.08 μL cyclohexane carboxylic acid as internal standard. The mixture was extracted for 20 sec with 1 mL freshly distilled petroleum ether. Of the petroleum ether layer, 1 μL was injected into the GC. To determine the valproate concentration in brain, one hemisphere was weighed and homogenized in distilled water (total volume of hemisphere and water, 3 mL). To 0.5 mL of the homogenate, 0.5 mL 6 *N* HCl was added and the mixture was extracted for 20 sec with 3 mL petroleum ether, containing 0.77 μL cyclohexane carboxylic acid. Two-tenths milliliter of the organic layer was diluted with 3 mL of petroleum ether and 1 μL of this solution was injected into the GC. The recovery of the extraction of valproate was in each instance 70%.

A Hewlett Packard Model 5710A gas chromatograph, equipped with a flame ionization detector and a split inlet injection system, was used. A fused silica capillary column (10 m × 0.53-mm ID) with a cross-linked FFAP stationary phase was used. Temperatures and chromatographic conditions were as follows: injection port, 250°C; oven, 200°C; detector, 140°C; helium as carrier at 20 mL/min through the column and 30 mL/min through the detector; hydrogen, 30 mL/min; and air, 270 mL/min. Retention times were 1.2 and 2.3 min for valproate and cyclohexane carboxylic acid, respectively. Chromatograms were recorded on a Hewlett Packard 3390A reporting integrator. Calibration curves were constructed in the concentration range between 10 and 800 mg · L^{-1} and were linear with $r > 0.996$. The detection limit was about 1 mg/L; coefficients of variation were less than 5% for plasma, plasma ultrafiltrate, and cerebrospinal fluid and less than 7% for brain tissue ($n = 5$).

Protein binding of valproate was determined by means of ultrafiltration using the Amicon MPS-1 system (Grace B.V., Rotterdam, The Netherlands).

Data Analysis

Pharmacokinetics/Pharmacodynamics

The plasma concentration versus anticonvulsant effect curves were constructed by combining the concentration versus time and anticonvulsant effect versus time profiles, obtained during continuous infusion of sodium valproate. At the time points where no blood samples were taken, concentrations were determined graphically by linear interpolation between the data points. The plasma concentration versus anticonvulsant effect curves were characterized on the basis of a least-squares spline function with a variable number of knots. The valproate concentrations needed to achieve an anticonvulsant effect of 200, 400, 600, 800, and 1000 μA were calculated.

In order to determine the equilibration kinetics of valproate between plasma and the central nervous system during the continuous infusion, a separate experiment was performed in rats with permanent cannulas in the cisterna magna (for serial sampling of the cerebrospinal fluid) and in the jugular vein (for infusion of valproate). In this experiment the concentration of valproate in simultaneously obtained samples of plasma and cerebrospinal fluid was determined during a continuous infusion of sodium valproate at the same rate as in the pharmacodynamic investigations. The results of this experiment showed that equilibrium conditions were reached at 30 min following the start of the infusion.

Statistics

Differences between age groups in clinical biochemical indices and in valproate concentrations needed to achieve an anticonvulsant effect of 200, 400, 600, 800, and 1000 μA were statistically evaluated by one-way analysis of variance with multiple comparison. *P* values lower than 0.05 were judged to be significant.

RESULTS

Clinical Biochemical/Pathological Evaluation

The results of the clinical biochemical indices are shown in Table I. The values of the clinical biochemical parameters are all within the normal limits for rats of these different age

groups of this particular strain of rats. At autopsy one 25-month-old animal showed moderate to severe renal cortical lesions (tubular atrophy, cast formation, moderate diffuse glomerulopathy). This rat was excluded from the calculations and the statistical evaluation.

Pharmacokinetic/Pharmacodynamic Evaluation

The baseline seizure threshold values (Table II) appeared to be independent of age. In Fig. 1 the plasma concentration versus anticonvulsant effect profile in a typical 3-month-old animal is shown. With increasing age a parallel shift of the concentration effect relationship to lower concentrations was observed (Fig. 2). The valproate plasma concentrations needed to achieve an anticonvulsant effect intensity of 800 and 1000 μA showed a statistically significant decrease of about 40% between the ages of 6 and 37 months (Table II). The concentrations needed to achieve an anticonvulsant effect of 200, 400, and 600 μA were also lower in elderly rats. This difference, however, did not reach statistical significance.

No clear age-related changes in the distribution of valproate between plasma, cerebrospinal fluid and brain tissue were observed as the values of the concentration ratios showed no statistically significant differences between the different age groups (Table III).

DISCUSSION

The mechanism of the anticonvulsant effect of valproate

Table I. Effect of Age on Selected Clinical Biochemical Indices[a,*]

	Age (months)						
	3	6	12	19	25	31	37
Number of animals	9	10	10	10	9	8	4
Body weight (g)	200 ± 9	309 ± 7[a]	362 ± 9[a,b]	416 ± 17[a,b]	439 ± 13[a,b,c]	380 ± 10[a,b,e]	368 ± 18[a]
Liver weight (g)	7.1 ± 0.4	7.6 ± 0.1	8.7 ± 0.3	9.4 ± 0.4 (n = 9)	10.3 ± 0.7	10.7 ± 1.1	9.5 ± 0.7
Liver wt/ body wt (%)	3.62 ± 0.24	2.47 ± 0.04	2.41 ± 0.08	2.30 ± 0.07 (n = 9)	2.37 ± 0.37	2.78 ± 0.22	2.57 ± 0.12
Blood aspartate aminotransferase (IU/L)	94.2 ± 7.6	100.7 ± 7.5	97.1 ± 8.1	96 ± 12	101 ± 17	73.5 ± 4.1	77.8 ± 8.8
Blood alanine aminotransferase (IU/L)	46.2 ± 2.0	41.0 ± 1.8	42.3 ± 1.7	34.1 ± 2.1[a]	32.4 ± 2.2[a,c]	36.5 ± 2.2	32.3 ± 2.1[a]
Plasma albumin (mg/mL)	35.2 ± 4.0	29.4 ± 1.6	29.9 ± 1.6	27.5 ± 1.3	27.2 ± 1.4	27.4 ± 1.8	28.4 ± 0.9
Plasma total protein (mg/mL)	72.9 ± 1.3	83.4 ± 2.8	80.5 ± 0.6	83.3 ± 1.5	79.9 ± 1.3	80.2 ± 0.7	76.3 ± 1.9
Blood glucose (mmol/L)	5.3 ± 0.3	5.4 ± 0.1	4.9 ± 0.2	5.2 ± 0.2	4.8 ± 0.2	5.4 ± 0.3	5.1 ± 0.3
Blood urea nitrogen (mmol/L)	8.0 ± 0.4	9.3 ± 0.3	8.1 ± 0.3	7.1 ± 0.3[b]	6.7 ± 0.4[b]	7.3 ± 0.2[b]	7.5 ± 0.3
Creatinine clearance (mL/hr · kg)	213 ± 16	171 ± 14	168 ± 11	154 ± 10	131 ± 14[a]	180 ± 20	138 ± 13
Urine production (mL/24 hr)	10.5 ± 1.2	10.1 ± 1.1	11.5 ± 0.4	7.9 ± 0.8[c]	8.6 ± 1.1	13.9 ± 1.9	9.2 ± 2.1
Osmolality (mOsm/L)	1690 ± 130	1940 ± 100	1541 ± 49	1810 ± 120	1700 ± 110	1340 ± 68[b]	1267 ± 41[b,c,d]

[a] Results are presented as mean ± SE.

* Superscripts: (a) significantly different from 3-month value, $P < 0.05$; (b) significantly different from 6-month value, $P < 0.05$; (c) significantly different from 12-month value, $P < 0.05$; (d) significantly different from 19-month value, $P < 0.05$; (e) significantly different from 25-month value, $P < 0.05$.

Table II. Effect of Age on Baseline Seizure Threshold Values and Pharmacodynamics of Valproate[a,*]

	Age (months)						
	3	6	12	19	25	31	37
Number of animals	9	10	10	10	8	8	4
Baseline seizure threshold (μA)	723 ± 89	674 ± 36	687 ± 83	686 ± 42	643 ± 89	723 ± 40	740 ± 56
$Conc._{200}$ (mg/L)[b]	592 ± 84	582 ± 42	511 ± 74	452 ± 52	459 ± 54	449 ± 43	353 ± 83
$Conc._{400}$ (mg/L)	862 ± 67	893 ± 58	802 ± 82	777 ± 50	713 ± 54	642 ± 40	505 ± 49[b]
$Conc._{600}$ (mg/L)	1048 ± 67	1073 ± 65	1000 ± 77 (n = 9)	986 ± 51	901 ± 65	786 ± 40	664 ± 37
$Conc._{800}$ (mg/L)	1163 ± 58	1204 ± 69	1153 ± 53 (n = 9)	1118 ± 54	1036 ± 78	889 ± 35[a,b,c]	744 ± 47[a,b,c,d]
$Conc._{1000}$ (mg/L)	1287 ± 47 (n = 7)	1329 ± 79	1303 ± 47 (n = 8)	1249 ± 62 (n = 9)	1160 ± 90	996 ± 33[a,c]	811 ± 62[a,b,c,d]

[a] Results are presented as mean ± SE.

[b] $Conc._{200}$, concentration needed to achieve an anticonvulsant effect consisting of an elevation in seizure threshold over baseline level of 200 μA.

* Superscripts: (a) significantly different from 3-month value, $P < 0.05$; (b) significantly different from 6-month value, $P < 0.05$; (c) significantly different from 12-month value, $P < 0.05$; (d) significantly different from 19-month value, $P < 0.05$.

is not completely understood, but it appears to be mediated at least in part through an interaction with GABAergic neurotransmission. Thus valproate is inhibits GABA degradation (9) and increases GABA release from nerve endings (15) and there appears to be a direct interaction with the GABA–benzodiazepine receptor complex (16).

An age-related decrease was found to occur in the sensitivity of the hypothalamic–pituitary system to valproate, resulting in decreased pituitary hormone secretion (10). Because GABA plays a role in the regulation of pituitary hormone secretion (17), it seems likely that the reduced hypothalamus–pituitary response with increasing age results from changes of GABAergic transmission (10).

To determine whether increasing age is also associated with a decrease in the sensitivity to the anticonvulsant effect of valproate, the drug was given as a slow intravenous infusion and the intensity of the anticonvulsant effect was measured repeatedly on the basis of the direct cortical stimulation model (11). Since also the plasma concentration of valproate was measured at several time points, concentration versus anticonvulsant effect profiles could be determined in individual animals. A nonlinear relationship between valproate concentration and anticonvulsant effect intensity was observed, with significant effects occurring in the concentration range between 300 and 1200 mg · L^{-1} and with no apparent maximal effect (Fig. 1). The concentrations at which the effect in our study occur are somewhat higher compared to those observed in a study by Löscher *et al.* (18) where the classical maximal electroshock test was used as the pharmacodynamic end point. A possible explanation for this difference is that in our rats the baseline seizure threshold was determined twice daily in the period of 2 weeks before the actual experiment took place. This repeated stim-

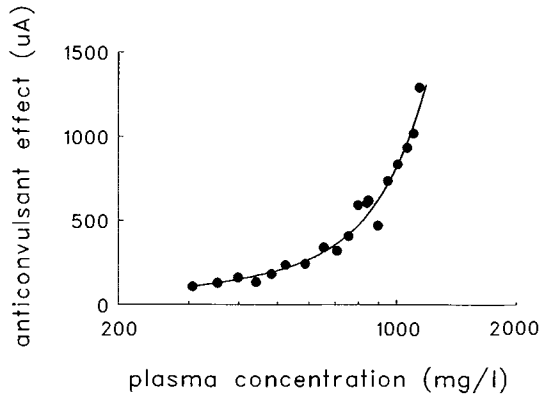

Fig. 1. Anticonvulsant effect vs plasma concentration profile measured during continuous sodium valproate intravenous infusion at a rate of 5.5 mg/min · kg in a typical 3-month-old animal. Anticonvulsant effect intensities (μA) are expressed as the elevation in seizure threshold over baseline level. The line through the data points represents the least-squares spline used for interpolation.

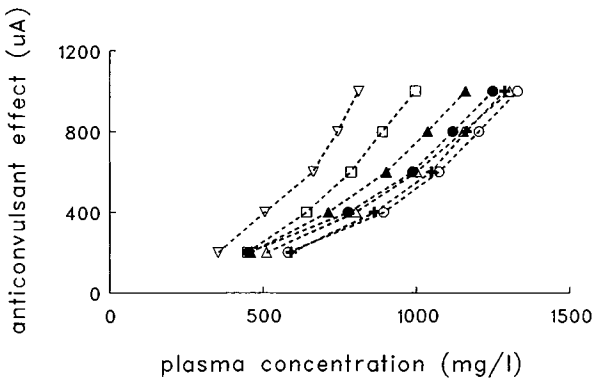

Fig. 2. Plasma concentrations of valproate required to achieve an elevation the threshold for localized seizure activity (TLS) of 200, 400, 600, 800, and 1000 μA for the different age groups: (+) 3 month old; (○) 6 month old; (△) 12 month old; (●) 19 month old; (▲) 25 month old; (□) 31 month old; (▽) 37 month old.

Table III. Effect of Age on Relative Distribution of Valproate[a]

	Age (months)						
	3	6	12	19	25	31	37
Number of animals	8	10	9	10	8	8	4
Conc. csf/conc. plasma free[b]	0.71 ± 0.02	0.73 ± 0.02	0.65 ± 0.02	0.77 ± 0.08	0.74 ± 0.01	0.75 ± 0.18	0.74 ± 0.08
Conc. csf/conc. plasma total	0.65 ± 0.02	0.67 ± 0.03 (n = 9)	0.61 ± 0.02 (n = 8)	0.66 ± 0.06 (n = 9)	0.71 ± 0.02 (n = 7)	0.65 ± 0.04 (n = 6)	0.67 ± 0.07
Conc. csf/conc. brain	1.86 ± 0.06 (n = 7)	2.08 ± 0.20 (n = 9)	2.12 ± 0.08 (n = 7)	1.88 ± 0.10 (n = 9)	1.94 ± 0.09 (n = 7)	1.95 ± 0.09 (n = 6)	2.40 ± 0.16
Plasma protein binding (%)	9.0 ± 1.9	10.7 ± 2.3	8.2 ± 2.3	13.9 ± 3.0	8.9 ± 2.6	9.4 ± 3.0	9.6 ± 0.9
Conc. brain/conc. plasma free	0.38 ± 0.01 (n = 7)	0.38 ± 0.03	0.31 ± 0.01	0.40 ± 0.03	0.39 ± 0.02	0.39 ± 0.02	0.31 ± 0.01
Conc. brain/conc. plasma total	0.34 ± 0.01 (n = 7)	0.36 ± 0.02	0.29 ± 0.01	0.34 ± 0.02	0.37 ± 0.02	0.34 ± 0.01	0.28 ± 0.01

[a] Results are presented as mean ± SE.
[b] conc., concentration; csf, cerebrospinal fluid.

ulation results in a decrease in the baseline seizure threshold, presumably as a result of some kind of "kindling" (11). It has been reported that "kindling" (which also occurs in epileptic patients as part of the pathophysiology of epilepsy) can be associated with a reduced sensitivity to actions of certain antiepileptic drugs, in particular drugs which act at the GABA–benzodiazepine receptor complex (19).

Between 6 and 37 months of age, a parallel shift in the concentration versus anticonvulsant effect relationship toward lower concentrations was found. This finding suggests an increased sensitivity of the brain to the anticonvulsant effect with increasing age.

In order to conclude that there is indeed a true change at the level of the pharmacodynamics, however, it is necessary to determine that there are no confounding pharmacokinetic factors that could account for the observed shift in the concentration effect relationship. Changes in distribution between plasma (where the valproate concentrations have been measured) and the site action and the role of possible active metabolites need to be considered. In the present investigation, altered distribution did not seem to be a contributory factor. Only data points obtained later than 30 min after the start of the infusion (at which time equilibrium between the concentrations in plasma and the CNS was attained) were included. For the various age groups no important differences in the equilibrium distribution among plasma, cerebrospinal fluid, and brain concentrations or in the degree of plasma protein binding were observed (Table III).

The metabolism of valproate is rather complex. Several metabolites have been identified, some of which possess pharmacological activity (20–22). This raises the question whether a change in metabolism may explain the shift in concentration–effect relationship. We do not think that this is the case. First, the concentration–effect relationships were determined during a continuous infusion of valproate rather than during the elimination phase following the administration of a bolus dose. As a result, the concentrations of the metabolites are probably low relative to those of the parent drug. Second, the intrinsic pharmacological activity of most metabolites appears to be low relative to that of valproate itself (21).

Thus an increase in the brain sensitivity to the anticonvulsant effect of valproate is the most likely explanation for the observed shift of the concentration effect relationship to lower concentrations. There is also evidence for an increased sensitivity to the effects of other antiepileptic drugs (23–27). The present finding is in contrast, however, with the effect of valproate on the hypothalamic–pituitary system, where a reduced sensitivity has been reported (10). Also, it conflicts with the observed reduced incidence of adverse effects in the elderly (2–4). This finding suggests that different mechanisms may be involved in the various effects of valproate, which are affected differentially by increasing age.

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