Cell Motility and the Cytoskeleton 40:220–237 (1998)

Cell Motility Is Inhibited by the Antiepileptic Compound, Valproic Acid and Its Teratogenic Analogues

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Valproic acid (VPA) is an established human teratogen that causes neural tube defects in 1–2 % of human foetuses exposed to the drug during early pregnancy. In this study, individual cell motility was evaluated using short- and long-term time-lapse video-recording and computer assisted image analysis, and it was found that VPA and selected VPA-analogues inhibited individual cell motility of L-cells in a dose-dependent manner. The compounds caused a decrease in the root-mean-square speed, S, and in the rate of diffusion, R, but an increase in the time of persistence in direction, P. Using short-term recordings and measurements of mean-cell speed, the reduction in the motile behaviour was shown to correlate with the teratogenic potency of the tested compounds. The observed effects of VPA on cell motility was independent of the employed L-cell clone, and could be reproduced in cells containing the neuronal marker NCAM and in the neuronal cell line N2a. Furthermore, the observed effect was independent of culture substratum, being observed for L-cells grown on fibronectin as well as on plastic. Immunofluorescence microscopy revealed that VPA-treatment of mouse L-cells caused a redistribution of F-actin and of a series of focal adhesion proteins, indicating that the effect of VPA on cell motility may be causally related to increased cell-substratum interactions or to alterations in the organisation or dynamics of the actin cytoskeleton. Cell Motil. Cytoskeleton 40:220–237, 1998.

Key words: cell speed; rate of diffusion; persistence time; fibroblast; L-cells; cytoskeleton

INTRODUCTION

The motile behaviour of eukaryotic cells plays a key role in many biological phenomena including embryogenesis, wound healing, immunological responses, and invasive and metastatic behaviour of cancer cells [Bronner-Fraser, 1993; Hauzenberger et al., 1997; McCawley et al., 1997; Van Roy and Mareel, 1992]. Therefore, studies on the effect of drugs and toxins on cell motility are of the utmost importance. In vitro studies of cell migration may be performed as cell population measurements, using, e.g., the under-agarose or Boyden chamber assays, or as measurements of the motility of individual cells [Lauffenburger and Lindermann, 1993]. So far, motility studies

Contract grant sponsor: EU-Programme on In Vitro Developmental Toxicology; Contract grant number: BI02-CT-93–0471; Contract grant sponsor: EU-Programme on Neuronal Development and Regeneration; Contract grant number: BI02–93–0260; Contract grant sponsor: Danish Medical Research Council; Contract grant sponsor: Danish Natural Science Council; Contract grant sponsor: Danish Biotechnology Programme; Contract grant sponsor: Danish Cancer Society; Contract grant sponsor: BgVV (ZEBET, Berlin); Contract grant sponsor: American Biogenetic Sciences (Copiague, NY); Contract grant sponsor: John and Birthe Meyer Foundation.

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Received 17 November 1997; Accepted 16 March 1998

have mainly employed cell population measurements [Cook and Hampton, 1997; Dickinson et al., 1993; Kim et al., 1995; Komazawa et al., 1995; Slaton et al., 1994; Welch et al., 1989; Yoshida et al., 1996], as determination of motility of individual cells requires special microscope equipment and customised computer software.

The anti-epileptic drug valproic acid (2-*n*-propyl pentanoic acid, VPA) is a known human teratogen causing *spina bifida aperta* in 1–2% of human foetuses exposed to the compound during early pregnancy [Robert, 1988]. The malformations produced by VPA are species dependent, neural tube defects being observed only in man and mouse [Narotski et al., 1994; Nau et al., 1991]. The mouse is therefore suitable for in vivo investigations of the teratogenic effects of VPA, and as a consequence a mouse model has been established for this purpose [Ehlers et al., 1992a]. Furthermore, VPA-analogues with varying teratogenic activity have been produced and evaluated extensively using the mouse as an in vivo model [Nau et al., 1991; Ehlers et al., 1992b; Hauck and Nau, 1989, 1992; Hauck et al., 1991, 1992; Elmazar et al., 1993; Andrews et al., 1995]. The molecular mechanisms behind the teratogenic effects of VPA are unclear, but VPA has been shown to possess marked anti-proliferative effects [Andrews et al., 1995; Martin and Regan, 1991] and to interfere with embryonic folate metabolism [Wegner and Nau, 1992].

We have recently demonstrated that treatment of cultured fibroblastoid mouse L-cells with VPA and selected VPA-analogues caused an increase in their mean cell area, the effects correlating to the teratogenic potency of the tested compounds [Berezin et al., 1996]. Although no straightforward relationship has been demonstrated between cell morphology and motility, these results indicate that VPA might be capable of modulating cellular motility, and that this effect might contribute to the teratogenic potency of the compound. In the present study we, therefore, tested the effects of VPA and selected teratogenic and non-teratogenic VPA-analogues for their effect on individual cell motility of culture cells. Using long-term time-lapse video-recording and image analysis it was found that VPA and VPA-analogues caused a dose-dependent decrease in cellular speed and an increase in the time of persistence in direction. Furthermore, using determination of mean-cell speed obtained from short-term evaluation of cell motility, it was found that the inhibitory effect of the compounds correlated statistically significantly with their teratogenic potency. By the use of confocal immunofluorescent microscopy, it was found that VPA-treatment not only caused an increase in the mean cell area, but also changed the organisation of F-actin and various focal adhesion proteins. These results indicate, that the observed effects of VPA on cellular motility might be causally related to changes in cell-substrate adhesion or to altered organisation or dynamics of the actin cytoskeleton and focal adhesions. Furthermore, the results indicate, that the effect of VPA on cell motility may contribute to the teratogenic potency of this compound.

MATERIALS AND METHODS

Cell Culture

The L929 cell line was obtained from the European Collection of Animal Cell culture. The mouse neuroblastoma cell line, Neuro-2a (N2a) was purchased from the American Type Culture Collection (Rockville, MD). L929 cells were transfected using the calcium phosphate coprecipitation method with a full-length cDNA encoding a transmembrane human isoform of the Neural Cell Adhesion Molecule (NCAM-140, NCAM-B), which was inserted in sense of the eukaryotic vector pHβ-Apr-1-neo [Meyer et al., 1995]. The NCAM-transfected cell line was designated LBN110. Control cell lines, designated LVN101 and LVN212, were transfected with the expression vector alone.

The cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Gibco BRL, Gjaithersburg, MD) supplemented with 10% v/v heat inactivated foetal calf serum (FCS, Gibco BRL), fungizone (2.5 µg/ml), penicillin (100 U/ml), and streptomycin (100 µg/ml). The cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 and passaged at least 4 times and no more than 15 times when assays were performed.

Synthesis of VPA-Analogues

VPA was purchased from Sigma (St. Louis, MO). (*E*)-2-en-VPA was from Destin, Hamburg, Germany. Synthesis of (±)-4-en-VPA was performed by alkylation of propylmalonic acid diethylester with an unsaturated bromo compound, 1-bromo-2-propene, followed by saponification and decarboxylation [de Moura Capos and do Amaral, 1965]. The pure enantiomers R- and S-4-yn-VPA were prepared as described by Hauck and Nau [1992].

Coating With Extracellular Matrix (ECM) Substrata

For experiments requiring coating with fibronectin, 35 mm diameter tissue culture dishes (Nunc, Roskilde, Denmark) were covered with 10 µg fibronectin (Sigma) in 1 ml sterile H_2O and dried overnight at room temperature in a flow bench.

Treatment of Cells With VPA and VPA-Analogues

Subconfluent cells were dislodged with 0.5 mg/ml trypsin, 0.2 mg/ml EDTA in a modified Puck's saline (Gibco BRL) and seeded on 35 mm diameter tissue culture dishes or 6 well tissue culture plates (Nunc). The cells were grown for 48 h in medium containing 25 mM

HEPES and 0, 0.12, 0.6, or 3.0 mM VPA or VPA-analogues. Since stock solutions of the compounds (3 M) were made in DMSO, control cells were tested in the presence of DMSO. In all cases, the final concentration of DMSO was 0.1% (v/v). For short-term recordings cells were plated at a density of 3.5 x 10^3 cells/cm^2. For long-term recordings control cells were plated at a density of 1.0–2.0 x 10^3 cells/cm^2. In long-term recordings, 4 different wells were monitored simultaneously.

Video-Recordings of Individual Cell Motility and Cell Morphology

For short-term recordings tissue culture dishes were placed on a thermostatically controlled stage (Lincam Scientific Instruments LTD, Surrey, UK) mounted on a Nikon Diaphot 300 inverted microscope equipped with phase contrast optics. The temperature in the culture dish was maintained at 34°C. Video-recordings of live cells was made using a black and white CCD video camera (Burle, Lancaster, PA) attached to the microscope. For determination of short-term individual cell motility, automated 512 x 512 pixel image acquisition and storage were performed at 5-min intervals over a period of 20 min, using an image processing software, LARPOP, obtained from Image House, Copenhagen, Denmark.

For long-term recordings, a microscope equipped as described above was used, with the modifications that a modified plexiglas incubator (Nikon, Japan) was mounted around the microscopic stage. The temperature inside the incubator was maintained at 35°C using a thermostatically controlled heating fan (DFA, Copenhagen, Denmark). Furthermore, a motorised stage was mounted on the microscope, allowing simultaneous recordings from many different microscopic fields. Before recordings, the lids on culture dishes were tightly sealed by means of autoclave tape and placed on the microscopic stage for approximately 30 min for equilibration. Images were recorded from 10–12 different fields/well at 15-min intervals for 10 h using the software PRIGRA (Protein Laboratory, Copenhagen, Denmark). During the time of recording, no change in pH was observed and condensation on the lower side of the lid of the culture dishes was insignificant.

Determination of Cell Morphology and Individual Cell Motility

For short-term measurements, cell contours from the stored images of the live cells were recognised semi-automatically by means of thresholding and binary transformation. This method allows determination of the borders of the cells and thereby of the position of the centroid, as well as the cell area according to Soll et al. (1988). For long-term measurements the positions of individual cells were determined by manual marking of the center of the nuclei. Centroids or nuclear centers from consecutive video frames were utilised to generate tracks of moving cells. Determinations of tracks and subsequent calculations were performed using the image processing software PRIMA (Protein Laboratory). In order to exclude the influence of cell-cell interactions on cellular motility and morphology, only single cells were evaluated. Furthermore, only non-dividing cells that did not leave the frames during the period of recording were included in the calculations. During long-term experiments, the amount of cells leaving the frames was negligible, whereas a large proportion (20–40 %) of the cells underwent mitosis.

Both short-term and long-term recordings were used for the calculation of mean-cell speed (designated S_τ, τ being the time-interval in minutes). S_τ is a time-interval dependent parameter defined as δ/τ, where δ is the mean distance of displacement of a cell within a certain time-interval, τ [Dunn, 1983].

Data from long-term recordings were expressed as the mean-square-displacement of the cells, $\langle d^2 \rangle$ in relation to τ, and fitted to the equation describing individual cell motility [Dunn, 1983; Gail and Boone, 1970]:

$$\langle d^2 \rangle = 2S^2P(\tau - P(1 - e^{-\tau/P}))$$

This equation is based on the assumption that the motile behaviour of cultured cells is a persistent random walk whose characteristics are determined by the theoretical parameters root-mean-square speed, S (distance/time), and persistence time of direction, P (time). Intuitively, the persistence time of direction can be described as the average time between significant changes in the directional movement of a single cell [Lauffenburger and Lindermann, 1993]. The root-mean-square speed is a mathematically derived determination of the speed of the cells, that unlike the mean-cell speed is independent of the chosen time-interval between observations. Also, the rate of diffusion, R (distance^2/time), was calculated, R being equal to $2S^2P$. When fitting experimental data to the theoretical curve, it was of importance to the accuracy of the estimations of P and S, that the observation time was considerably longer than P, ensuring that the curve had reached its linear part. The chosen cell line turned out to have a relatively long persistence time, and, therefore, to improve the accuracy of the estimations of P, S, and R, the $\langle d^2 \rangle$-values were calculated using the method of overlapping intervals (e.g., a given $\langle d^2 \rangle$-value at time-interval τ minutes is the mean $\langle d^2 \rangle$-value of all τ minute intervals; thus if τ = 60 min, the employed intervals were 0–60 min, 15–75 min, 30–90 min, etc.).

The amount of cells used for calculations in the individual long-term experiments varied between 34 and

123, the mean being 75. In 7 out of 48 experiments, the cells in a single well exhibited a significant decrease in motility in the last 1 to 2 h of the recording. In these cases, the data from the last 1 to 2 h were excluded.

Immunofluorescence Microscopy

Cells untreated or treated with 3 mM VPA, grown on four well LabTek glass slides (Nunc) were fixed in 3 % (w/v) paraformaldehyde in PBS for 10 min, permeabilised with 0.2 % (v/v) Triton X-100 in PBS for 2 min, and washed in PBS containing 1% BSA. They were then incubated for 60 min with mouse monoclonal antibodies against phosphotyrosine (PY20), Focal Adhesion Kinase (FAK), paxillin (all three antibodies from Transduction Laboratories, Lexington, KY), or vinculin (Sigma) diluted 1:25 in PBS containing 10% rabbit serum and 0.01% Triton X-100. After washing, the cells were incubated for 30 min with a rabbit anti-mouse Ig-FITC conjugate (DAKO, Glostrup, Denmark) diluted 1:25. β-tubulin staining was performed using fixed cells incubated with a mouse monoclonal antibody against β-tubulin (Amersham, Hørsholm, Denmark) diluted 1:20 in PBS containing 10% goat serum, followed by incubation with a goat anti-mouse Ig-FITC conjugate (Calbiochem, La Jolla, CA). Filamentous actin (F-actin) was stained using Texas Red X-conjugated phalloidin (Molecular Probes, Eugene, OR) diluted 1:30 in PBS.

Cells were mounted using Slowfade (Molecular Probes) and viewed using a MultiProbe 2001 Laser Scanning Confocal Microscope with an argon/krypton laser (Molecular Dynamics) and with a 60x objective (1.4 numerical aperture). FITC-stained cells were scanned using a wavelength of 488 nm, a 510 nm primary dichroic beamsplitter, and a 510 nm long-pass barrier filter. Texas Red X-stained cells were scanned using a wavelength of 568 nm, a 488/568 primary dichroic beamsplitter, and a 590 nm long-pass barrier filter. All scannings were performed using a pinhole size of 100 µm. Pixel images (1024 x 1024) with pixel sizes of 0.11 or 0.21 μm^2 were scanned as single sections taken close to the cell-substratum interface.

Statistics and Graphical Presentations

Statistics and graphical presentations were performed using PRIMA (Protein Laboratory) ''Statistica'' v4.5 (Statsoft, Tulsa, OK), ''Fig. P'' v2.2 (Biosoft, Cambridge, UK), and ''Excel'' v5.0 (Microsoft).

Estimations of IC_{50}-values of the effect of VPA and various VPA-analogues on S, R, and S_τ were based on the visual reading of log concentration-response curves. Shortly, for each selected drug-dose, the normalised mean-value ± SEM of the parameter of interest was plotted on a log dose-scale. The individual mean- and mean ± SEM-values were connected with straight lines, and the drug-concentration at which the lines intercepted the 50% inhibition line was determined visually.

RESULTS

VPA Inhibits Cell Motility in a Dose-Dependent Manner

Previous reports have demonstrated that exposure to VPA in a number of cell lines causes dramatic changes in cell morphology in a dose and time dependent manner, the most prominent change being a strong increase in cell area [Berezin et al., 1996]. Although no straightforward relationship has been demonstrated between cell morphology and cell motility these results indicate that VPA might affect the latter parameter.

In order to test the effect of VPA on cell motility long-term recordings of individual cells were performed using mouse fibroblastoid L-cells (clone LVN101) treated with different concentrations of VPA (0.12, 0.60, and 3.0 mM) for 48 h. Cell cultures were transferred to the microscope where images from a number of different areas were recorded with 15 min intervals for a total recording period of 10 h.

Figure 1 shows data from a representative experiment. The tracks of individual cells from 10–12 different microscopic fields superimposed into one frame are shown. It can be seen that control cells exhibited varying modes of motile behaviour, the majority of the cells being very motile, thereby producing long tracks. At increasing concentrations of VPA, however, a clear decrease in motility could be observed as reflected by a decrease in the length of the tracks, resulting in a high amount of almost immobile cells at the highest dose of VPA (3.0 mM). We, therefore, conclude that VPA inhibits cell motility of the employed clone of L-cells in a dose-dependent manner.

Teratogenic VPA-Analogues Cause a Stronger Inhibition of Cell Displacement Than Non-Teratogenic Analogues

The effect of VPA on cell morphology has been shown not only for VPA, but also for VPA-analogues, the effect correlating with the teratogenic potency of the individual compounds [Berezin et al., 1996]. In order to examine whether the effect of VPA on cell motility might also be reproduced by VPA-analogues, and whether the effect might correlate to the teratogenic potency of the individual compounds, VPA and VPA-analogues with identical anticonvulsive effects but different teratogenic potencies were investigated for their effect on cell displacement. L-cells were treated with VPA, (*E*)-2-en-VPA or (±)-4-en-VPA at a concentration of 3.0 mM for 48 h. The teratogenic potency of these compounds has

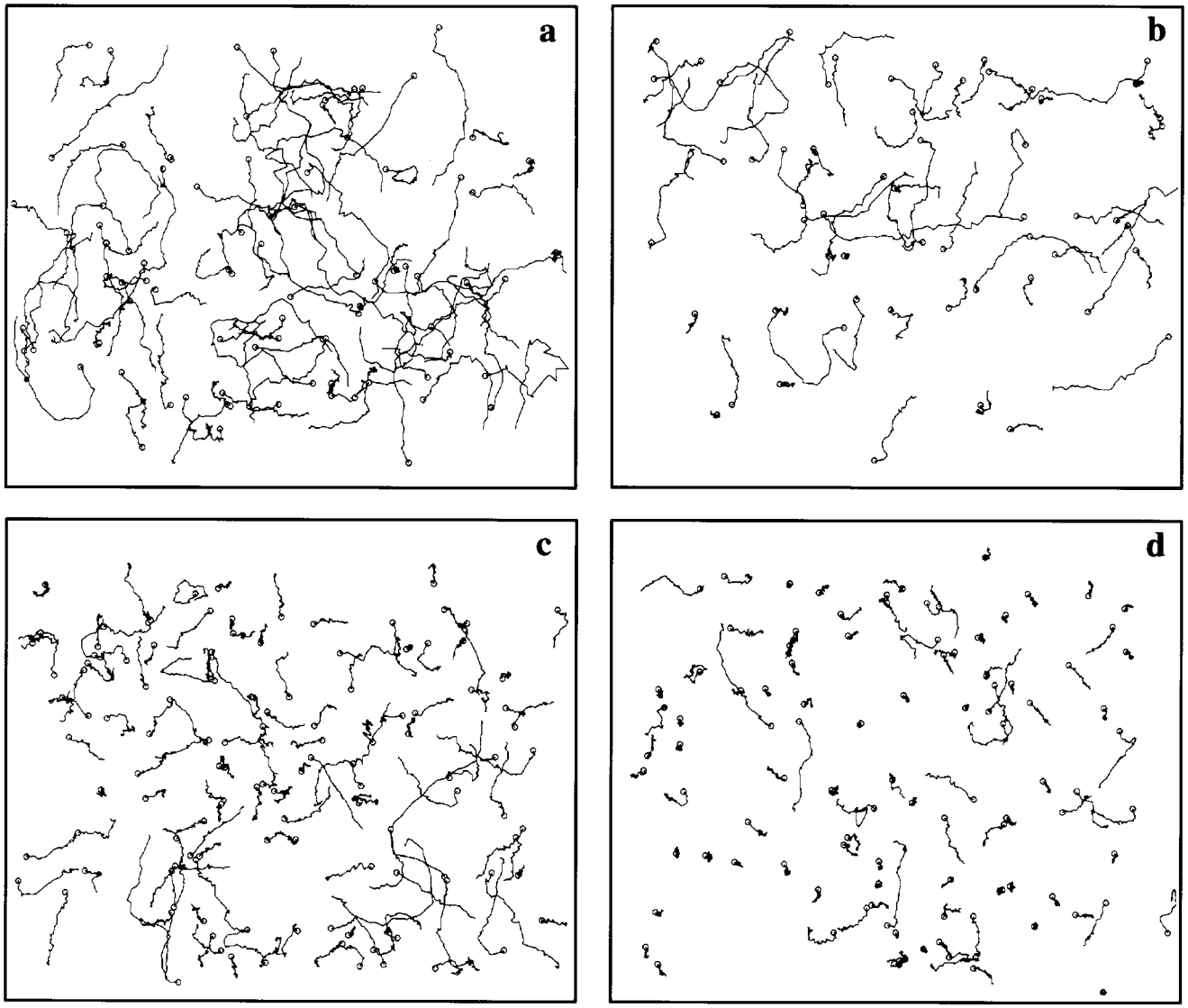
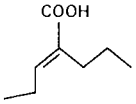
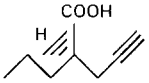
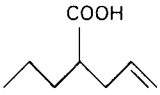
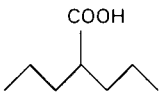
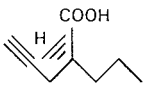


Fig. 1. Tracks showing the migration of LVN101 cells from 48 h cultures treated with different concentrations of VPA; control (**a**), 0.12 mM VPA (**b**), 0.60 mM VPA (**c**), and 3.0 mM VPA (**d**). Each frame contains superimposed tracks from a number of different fields from the same culture dish. Each track represents the motile behaviour of a single cell recorded for 10 h with 15-min intervals. The number of cells in each frame are 85 (control), 58 (0.12 mM VPA), 123 (0.60 mM VPA), and 102 (3.0 mM VPA).

been estimated previously by measuring the rate of exencephaly induced in living mice foetuses [Nau et al., 1991], see Table I. Whereas (±)-4-en-VPA has a teratogenic activity only slightly lower than VPA, (*E*)-2-en-VPA does not induce exencephaly, although it has been demonstrated to cause neurobehavioral alterations in prenatally exposed rats [Fisher et al., 1994]. In Figure 2, the results from representative experiments are shown as windrose diagrams. Each windrose shows the tracks of all cells from 10–12 microscopic fields from a single experiment. However, in contrast to the presentation shown in Figure 1, the starting points of all cell tracks have been superimposed. From Figure 2 it can be seen, that VPA as well as the two VPA-analogues caused a severe reduction in the overall displacement of the cells. After 10 h of observation, control cells exhibited a mean-square-displacement, $\langle d^2 \rangle$, of 10,437 μm^2, whereas cells treated with (*E*)-2-en-VPA, (±)-4-en-VPA and VPA had $\langle d^2 \rangle$-values of only 2,875, 1,355, and 890 μm^2, respectively. Thus, the migratory behaviour of the cells was affected by all three drugs, and the two teratogenic compounds (±)-4-en-VPA and VPA caused a stronger decrease in cell displacement than the non-teratogenic analogue (*E*)-2-en-VPA, indicating that the effect of these compounds on cell

TABLE I. Structure and Teratogenic Potency of VPA and Selected VPA-Analogues*

	Name				
	E-2-en-VPA	R-4-yn-VPA	(±)-4-en-VPA	VPA	S-4-yn-VPA
Structure					
Exencephaly rate (% of living fetuses)	0	1	35	44	65

*Exencephaly rate was determined by Nau et al. [1991] using a dose of 3.0 mmol/kg of the individual compounds except for S-4-yn-VPA of which only 1.05 mmol/kg was administered.

motility might be partially related to their teratogenic potentials.

VPA and Its Analogues Cause a Dose-Dependent Decrease in Root-Mean-Square Speed, S, and Rate of Diffusion, R, and a Dose-Dependent Increase in Persistence Time, P

The results from the long-term studies of cell motility visualised as tracks or windroses demonstrated that VPA and its analogues caused a decrease in cell motility, but did not clarify whether this was due to changes in cell speed, persistence time, or both. Nor did the experiments give any information as to whether the observed differences between the tested compounds could be reproduced over a range of concentrations, or only observed at the employed concentration of 3.0 mM. Therefore, dose-response experiments of VPA, (±)-4-en-VPA and (*E*)-2-en-VPA using long-term recordings were performed.

Cells were exposed to each of the three compounds at three different concentrations, 0.12, 0.60, and 3.0 mM, and motility was recorded as described above. Subsequently, the cell-displacement was expressed as the mean-square-displacement, $\langle d^2 \rangle$, for $\tau = 0$–600 min, using the method of overlapping intervals. Figure 3a shows a representative dose-response experiment of VPA. It is seen that the drug caused a decrease in $\langle d^2 \rangle$ at increasing concentrations. By fitting the individual curves to the equation $\langle d^2 \rangle = 2S^2P\ (\tau - P(1 - e^{-\tau/P}))$ values of the root-mean-square speed, S, the persistence time, P, and the rate of diffusion, $R = 2S^2P$ was estimated (Fig. 3b). It is seen, that VPA caused a significant dose-dependent decrease in S and R, but an increase in P.

Table II summarises the values of S, R, and P at different concentrations of the three investigated drugs. All three drugs caused a decrease in S and R in a dose-dependent manner. Dose-response curves showing the effect of VPA on S and R gave IC_{50}-values of 0.90 mM (for S) and 0.50 mM (for R) (Fig. 4a and b, respectively). The IC_{50}-values of S and of R for the two VPA-analogues were not significantly different from those observed after VPA-treatments.

VPA and the two VPA-analogues also caused a dose-dependent increase in the persistence time relative to control cells. Whereas control cells had an average P of 139 minutes, P increased up to 298 min after drug treatment (Table II).

Mean-Cell Speed, S_τ, Can Be Correlated to the Teratogenecity of VPA and Its Analogues

Long- and short-term time-lapse video-recordings may also be used for the calculations of the mean-cell speed of the cells (S_τ) using different intervals (τ) between observations. In contrast to the root-mean-square speed, S, the mean-cell speed, S_τ, is a time-interval-dependent parameter. As τ approaches 0 min, S_τ approaches the instantaneous velocity of the cells [Dunn, 1983]. For $\tau > 0$, S_τ is a measure of both persistent (directional) and random cell motility.

IC_{50}-values for the theoretically derived parameters S and P had been unable to reveal differences in the inhibitory effect on cell motility caused by the tested drugs. In order to investigate whether S_τ was a more sensitive parameter, it was decided to recalculate the data obtained from the previous long-term recordings, and express them as S_τ.

For all three compounds investigated, the observed decrease in S had been accompanied by an increase in P. Thus, the decrease in speed caused by the drugs is to some extent counteracted by a, on average, more directional motile behaviour. In order to reduce the influence of persistency on the calculations of S_τ, we chose to calculate IC_{50}-values for S_τ using the shortest possible time-interval, $\tau = 15$ min, for all three compounds.

As for the root-mean-square speed (Fig. 4a), a decrease in the mean-cell speed, S_τ was observed at increasing concentrations of the drugs. Figure 5 shows a dose-response curve of the effect of VPA on S_τ. The IC_{50}-values of VPA on S_{15} was 2.1 mM, which was higher than the IC_{50}-values of VPA on root-mean-square speed, S, (0.90 mM, Fig. 4a). In Table III the IC_{50}-values for the three investigated compounds are given in relation to S_{15}. As was the case for the IC_{50}-values for the effect of the

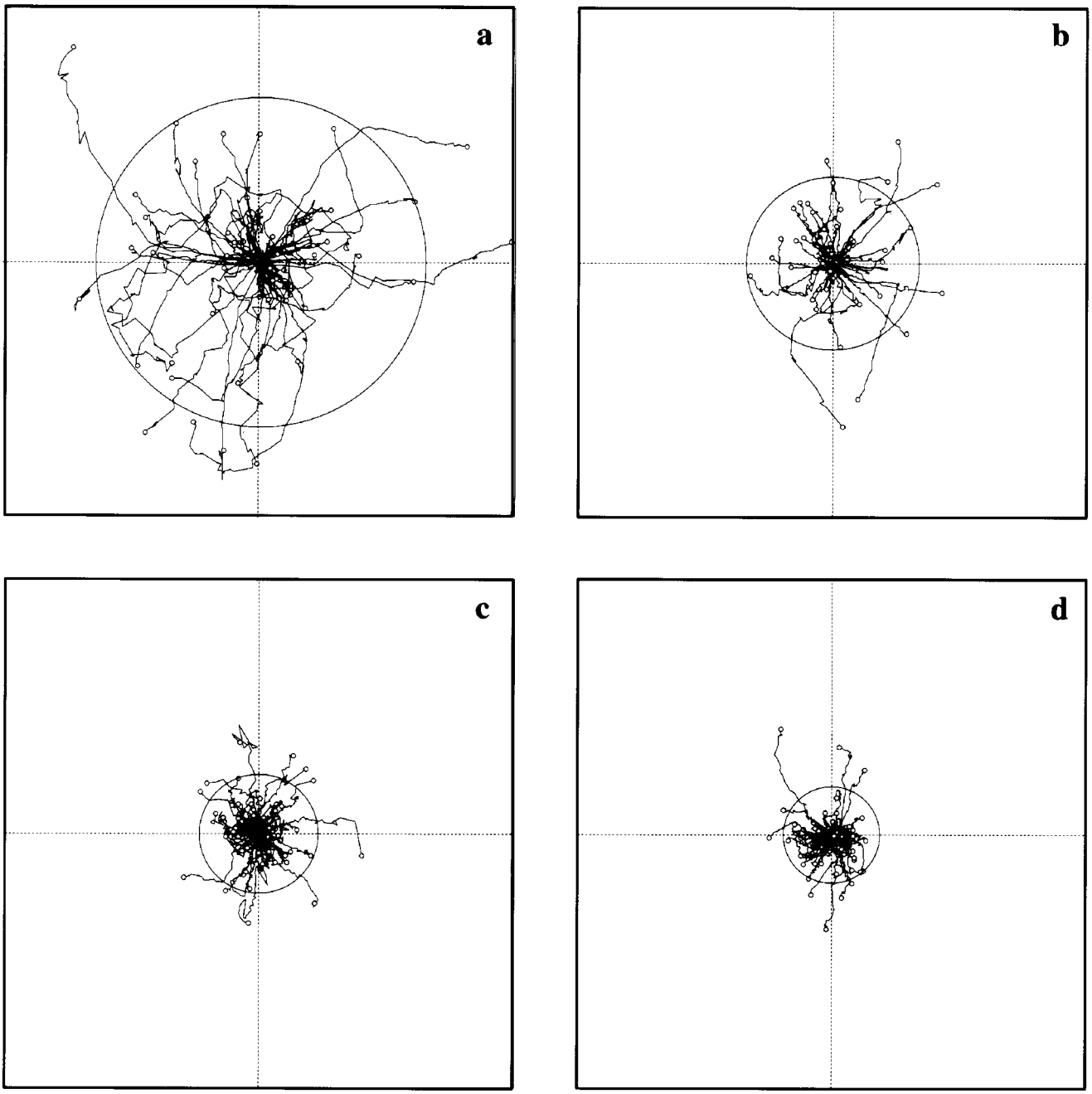


Fig. 2. Windrose presentations of 48-h LVN101 cell cultures. Each windrose contains tracks of single cells from 10–12 different fields from the same culture dish, where the starting point of all tracks has been superimposed. Each track represents the motile behaviour of a single cell recorded for 10 h with 15-min intervals. Control (72 cells) (**a**) and cells treated with 3 mM of (*E*)-2-en-VPA (72 cells) (**b**), (±)-4-en-VPA (95 cells) (**c**), or VPA (102) (**d**) are from different experimental days in which the displacement of control cells was not significantly different. The circle in each frame marks the $\sqrt{\langle d^2 \rangle}$ of the recorded cells.

compounds on the root-mean-square speed, S, none of the IC_{50}-values for S_{15} were significantly different from each other. However, when comparing the effect of the three compounds it can be seen that whereas the IC_{50}-value for (*E*)-2-en-VPA was above the employed drug concentration range, (±)-4-en-VPA and VPA had IC_{50}-values within the tested concentration range, the value for VPA being lower. Thus, the data indicate that there, indeed, might be a correlation between the inhibition of cell speed and the teratogenic potency of the individual compounds,

TABLE II. Root-Mean-Square-Speed, S, Rate of Diffusion, R, and Persistence Time, P, for LVN101 Cells Treated for 48 h With Different Concentrations (0.12, 0.6, and 3.0 mM) of (E)-2-en-VPA, (±)-4-en-VPA, and VPA*

Compound	Mean cell number	Number of experiments	S (μm/min)	R (μm^2/min)	P (min)
Control	69	(11)	0.2563 ± 0.021	17.54 ± 2.04	139 ± 16
(E)-2-en-VPA (mM)					
0.12	66	(4)	0.2230 ± 0.024	12.36 ± 1.46	128 ± 12
0.60	61	(3)	0.1739 ± 0.068	11.86 ± 4.75	215 ± 53
3.00	50	(3)	0.0793 ± 0.021	3.68 ± 1.92	260 ± 20
(±)-4-en-VPA (mM)					
0.12	84	(4)	0.1738 ± 0.019	11.07 ± 3.33	169 ± 28
0.60	75	(3)	0.1646 ± 0.033	11.90 ± 2.77	264 ± 104
3.00	83	(4)	0.0960 ± 0.015	3.77 ± 0.52	229 ± 50
VPA (mM)					
0.12	81	(4)	0.1793 ± 0.009	9.04 ± 2.00	135 ± 18
0.60	100	(3)	0.1640 ± 0.065	8.46 ± 2.92	217 ± 69
3.00	89	(3)	0.0619 ± 0.010	2.37 ± 0.66	298 ± 14

*Results are given as mean ± SEM calculated on the basis of experiment numbers given in the table.

but that the effect of the drugs were too similar to be distinguished using IC_{50}-values.

Whether a correlation between the inhibition of cell motility and the teratogenic potency of the individual compounds could, indeed, be demonstrated, was subsequently investigated for cells treated with the following compounds: (*E*)-2-en-VPA, R-4-yn-VPA, (±)-4-en-VPA, VPA and S-4-yn-VPA, all having identical anti-convulsive effects, but varying levels of teratogenic potentials (Table I).

Rather than measuring IC_{50}-values, it was decided only to employ one concentration of the compounds, 3 mM. Furthermore, the time-interval of observation was reduced in order for the measurements of S_τ to approximate the instantaneous velocity of the cells. Thus, S_τ was determined for $\tau = 5$ min (S_5) using a total observation time of only 20 min. From Figure 6 it can be seen that the non-teratogenic compounds (*E*)-2-en-VPA and R-4-yn-VPA did not influence S_5 significantly, whereas the three teratogenic compounds (±)-4-en-VPA, VPA, and S-4-yn-VPA inhibited mean-cell speed statistically significantly when compared to control cells (Fig. 6a). Furthermore, the effects of (±)-4-en-VPA, VPA, and S-4-yn-VPA were significantly different from each other ($P < 0.01$ for VPA vs. (±)-4-en-VPA and VPA vs. S-4-yn-VPA; $P < 0.001$ for (±)-4-en-VPA vs. S-4-yn-VPA). The capacity of the individual compounds for induction of exencephaly in vivo correlated statistically significantly to their effects on mean-cell speed ($r = -0.9502$, $df = 4$, $P < 0.01$) (Fig. 6b). Thus, under the chosen conditions, the change in mean-cell speed correlated inversely with the teratogenic potency of VPA and its analogues.

Not only did the teratogenic compounds significantly reduce cellular speed, but as previously reported they also induced a strong increase in cellular areas [Berezin et al., 1996]. In Figure 7a, it can be seen that the most pronounced effect on cellular area was observed after treatment with the strongly teratogenic compounds S-4-yn-VPA, VPA, and (±)-4-en-VPA. However, whereas the non-teratogenic compound R-4-yn-VPA had no effect, (*E*)-2-en-VPA caused a small but statistically significant increase ($P < 0.001$) in cellular area when compared to control cells. From Figure 7b, it also appears that S_5 of the treated cells was negatively correlated to the areas of the same cells ($r = -0.9459$, $df = 4$, $P < 0.01$). Thus, the teratogenic effect on cell morphology and cell speed may be causally related, or alternatively may be caused by the same initial teratogenically related alteration in cellular function.

Inhibition of Cell Motility by VPA Is Not Cell Type or Substratum Specific

In order to investigate the cellular specificity of the inhibition of cell motility by VPA, a number of experiments were performed in which S_5 of cells untreated or treated with 3 mM VPA for 48 h was calculated on the basis of short-term recordings as described above.

In order to ensure that the inhibitory effect of VPA was independent of the employed cell clone, the motility of another vector-transfected clone of L-cells, LVN212, was investigated. As shown in Figure 8, VPA caused approximately a 30% decrease in S_5 of LVN212, an inhibition similar to what had been obtained in clone LVN101 (Fig. 6a). Furthermore, the motility of untreated cells from the two clones was not significantly different (0.66 μm/min for LVN101 and 0.68 μm/min for LVN212). Thus, the decrease in mean-cell speed observed upon

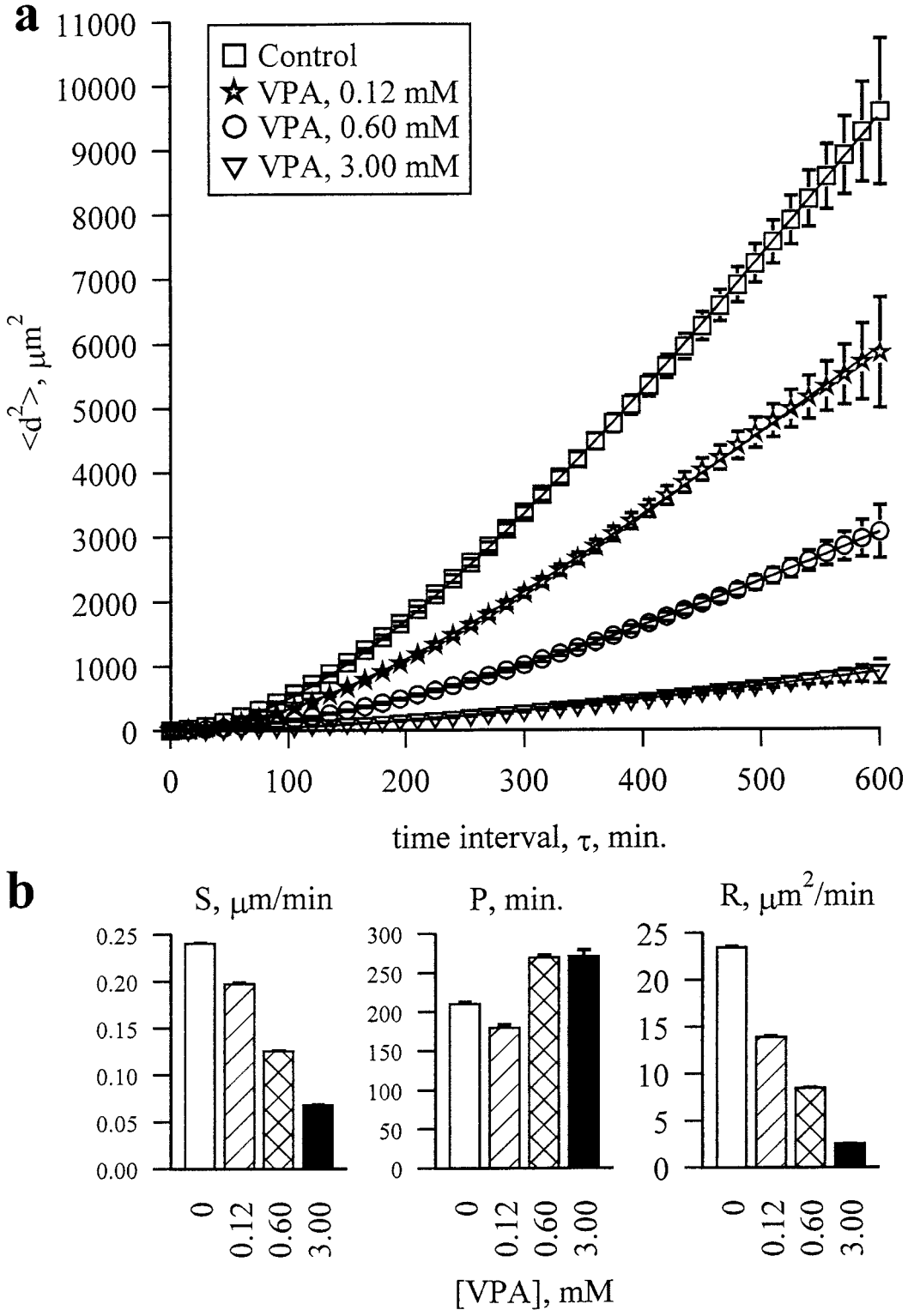


Fig. 3. **a:** Dose-response curves from an experiment on cultures of LVN101 cells treated for 48 h with different concentrations of VPA (0.12, 0.60, and 3.0 mM). For each concentration 10–12 different microscopic fields were recorded for 10 h with 15-min intervals. The number of cells in the individual experiments were 85 (control), 58 (0.12 mM), 123 (0.60 mM), and 102 (3.0 mM). Data points are expressed as $\langle d^2 \rangle \pm$ SEM based on cell numbers obtained by the method of overlapping intervals (Materials and Methods). Lines indicate curve-fitting to the equation $\langle d^2 \rangle = 2S^2P\ (\tau - P(1 - e^{-\tau/P}))$. **b:** Bar-diagrams showing the estimated values of the root-mean-square speed, S, the persistence time, P, and the rate of diffusion, R, based on the curve-fittings shown in Figure 3a. Error bars = the standard deviation of the estimates based on the goodness of fit for curve-fitting (d.f. = 39).

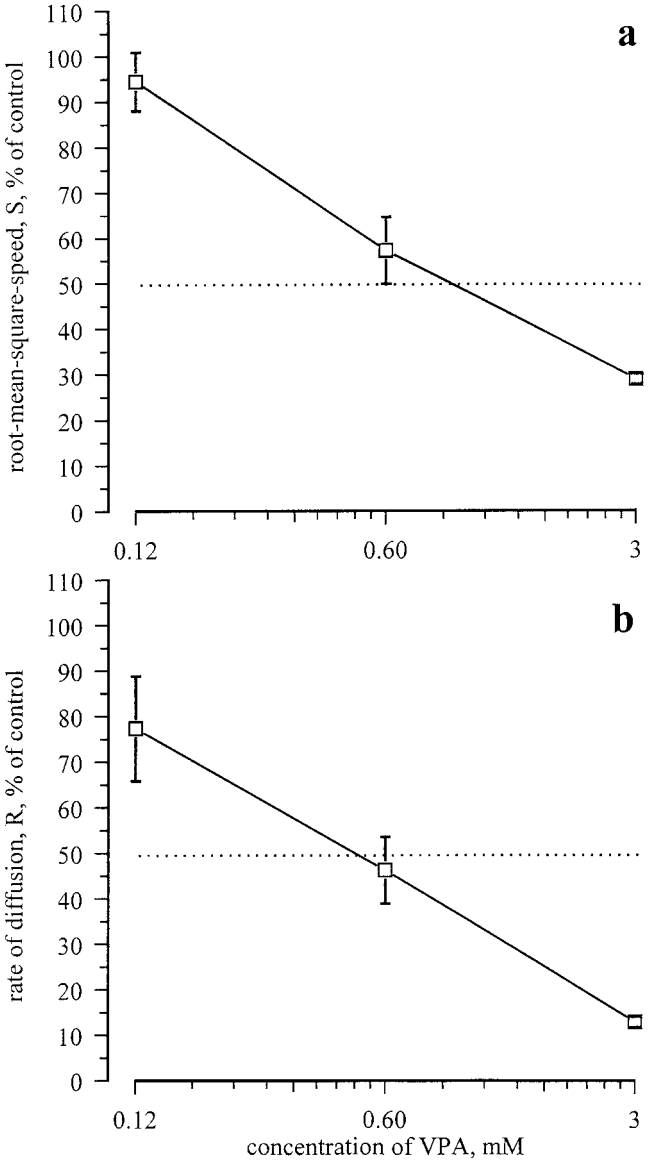


Fig. 4. VPA dose-response curves for estimated values of the root-mean-square speed, S (**a**) and the rate of diffusion, R (**b**). Forty-eight-hour cultures of LVN101 cells treated with different concentrations of VPA (0.12, 0.60, and 3.0 mM) were recorded for 10 h with 15-min intervals. Estimations of S and R were performed as described in Materials and Methods and Figure 3. Values of S and R are normalised in relation to the values obtained for untreated cells tested simultaneously. The mean number of cells used in the experiments for each concentration are given in Table II. The dotted lines indicate a 50% reduction of S and R, respectively. Bars = SEM based on experiment number (Table II).

TABLE III. Determination of IC_{50} of Mean-Cell-Speed, S_τ, for LVN101 Treated With (E)-2-en-VPA, (±)-4-en-VPA, and VPA, Using $\tau = 15$ Min*

Compound	IC_{50} of mean-cell-speed mean ± SEM (mM)	
(E)-2-en-VPA	>3[a]	
(±)-4-en-VPA	2.43	−0.3 +0.4
VPA	2.10	−0.02 +0.5

*Cell cultures treated with different concentrations of the drugs (0.12, 0.6, and 3.0 mM) for 48 h were recorded for 10 h as explained in the text. Results are given as mean ± SEM. ND, not detectable (outside tested concentration range).

[a](E)-2-en-VPA at 3 mM caused an inhibition of 53% when compared to control. SEM calculated on the basis of the number of experiments was ±3%.

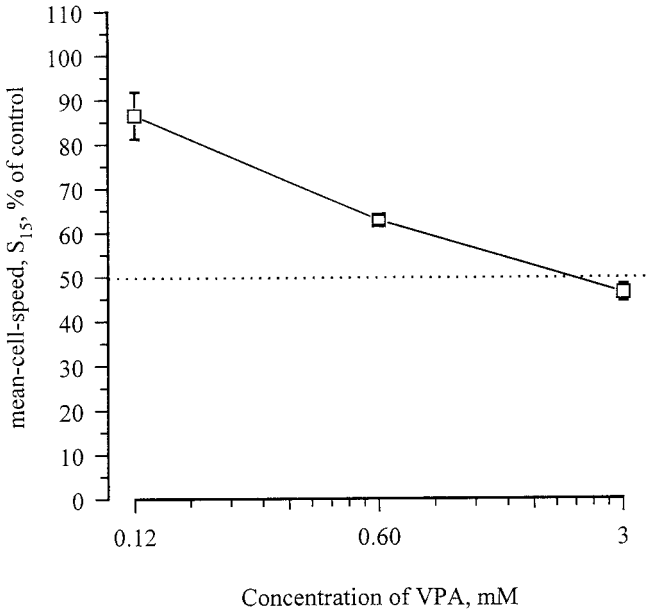


Fig. 5. VPA dose-response curve for determined values of the mean-cell speed, S_τ, using $\tau = 15$ min. Forty-eight-hour cultures of LVN101 cells treated with different concentrations of VPA (0.12, 0.60, and 3.0 mM) were recorded for 10 h with 15-min intervals. All values are normalised to the value obtained the same day for control cells. The mean number of cells used in the experiments for each concentration is given in Table II. The dotted line indicates a 50% reduction of S_τ. Bars = SEM based on experiment number (Table II).

exposure to VPA seemed to be independent of the chosen clone of L-cells.

As the most severe teratogenic effects of VPA are malformations of the nervous system, it was of interest to determine whether the effect of VPA on cell motility could be replicated in cells either containing a neuronal cell adhesion molecule (CAM), or being neuroepithelial by origin. One of the most widespread neuronal CAMs is the Neural Cell Adhesion Molecule, NCAM, a strongly expressed plasma membrane receptor involved in homo- and heterophilic cell-cell, and cell-substratum interactions, and in modulation of neurite outgrowth [reviewed in Rønn et al., 1997].

In order to investigate whether the presence of NCAM modified the VPA-induced alterations of cell motility, a human isoform of NCAM was transfected into

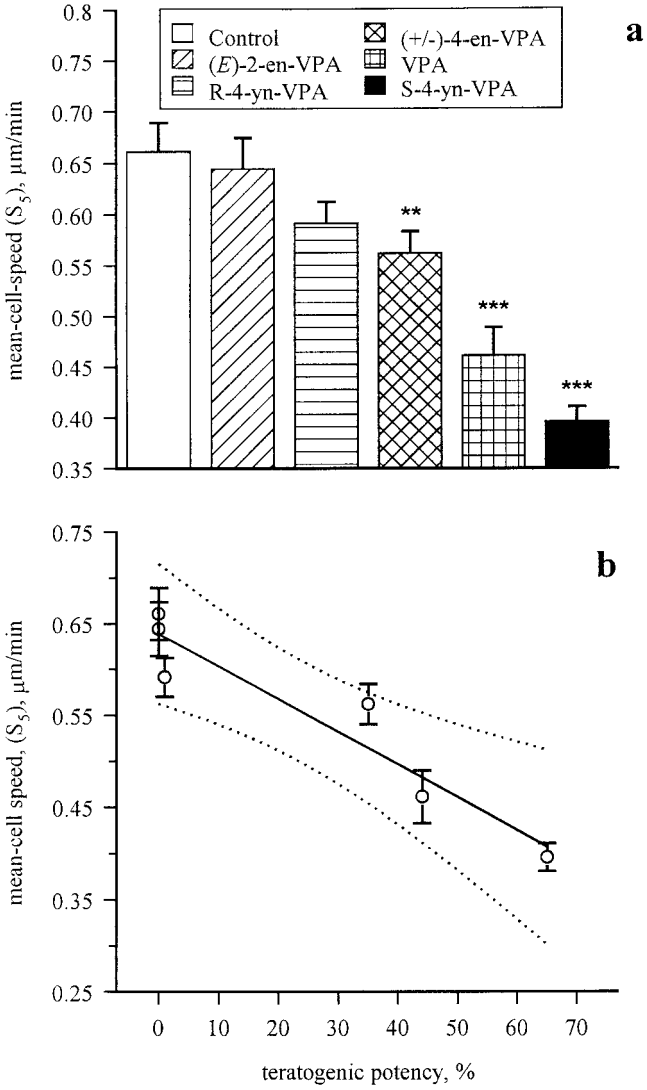


Fig. 6. **a:** The effects of treatment with 3 mM of VPA and various VPA-analogues on the mean-cell speed (S_5) of 48-h cultures of LVN101. Time-lapse video-recordings were performed at 5-min intervals over a period of 20 min. S_5 was estimated by determination of the rate of centroid translocation (Materials and Methods). $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to control cells, using a two-sided Student's *t*-test. **b:** Regression line and 95% confidence limits illustrating the correlation between exencephaly rates in vivo (expressed as % in live foetuses, compare Table I) of the employed VPA-analogues and mean-cell speed (S_5) displayed by the drug-treated LVN101 cells. The number of cells varied from 120 to 150 in the individual experiments. Results are given as mean ± SEM based on cell number.

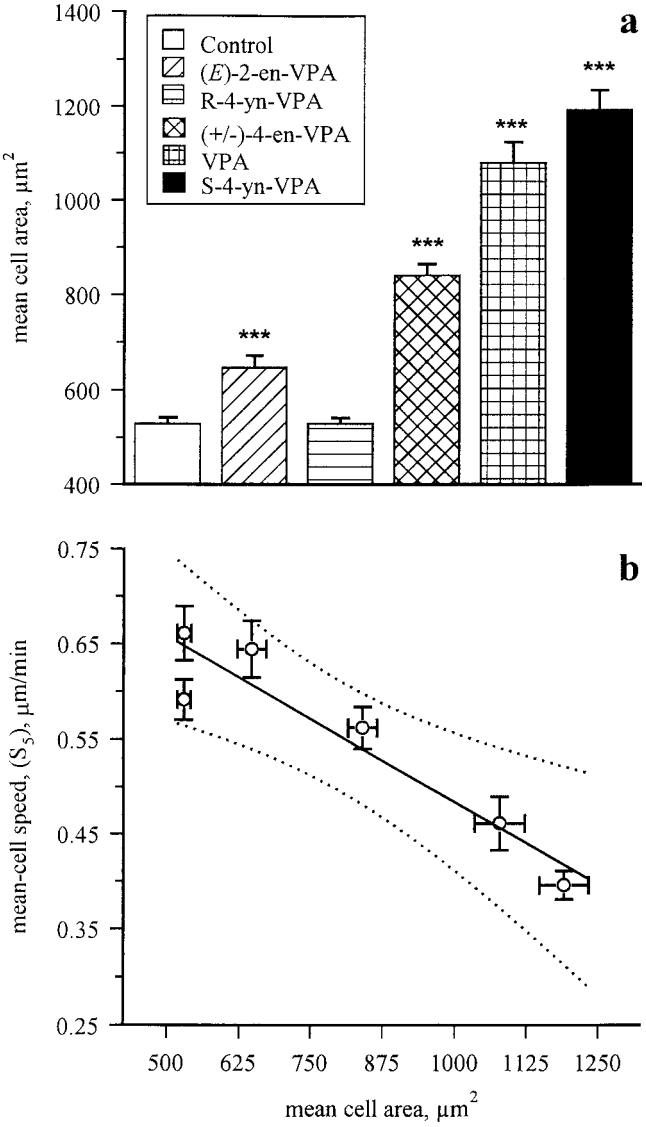


Fig. 7. **a:** The effects of treatment with 3 mM of VPA and various VPA-analogues on the mean cell area of 48-h cultures of LVN101. $^{***}P < 0.001$ when compared to control cells, using a two-sided Student's *t*-test. **b:** Regression line and 95% confidence limits illustrating the correlation between (S_5) and the mean cell areas. The number of cells varied from 120 to 150 in the individual experiments. Results are given as mean ± SEM based on cell number.

L-cells, generating the clone LBN110. As shown in Figure 8, the motility of untreated LBN110 cells was not significantly different from vector-transfected clones, and exposure of LBN110 cells to 3 mM VPA for 48 h caused a 48% reduction in S_5. Furthermore, when investigating the motility of a mouse neuronal cell line, N2a, a slightly higher motility was observed for untreated neuronal cells, as compared to untreated L-cells, and a significant reduction in S_5 (26%) was seen after 48 h of exposure to 3 mM VPA. Together these results indicate that the inhibition of cell motility by VPA was unaffected by the presence of the neuronal marker NCAM, and could be observed in cells of neuroepithelial as well as fibroblastoid origin.

The increase in mean cell area observed upon exposure of L-cells to VPA (Fig. 7a) has previously been demonstrated to be independent of the culture substra-

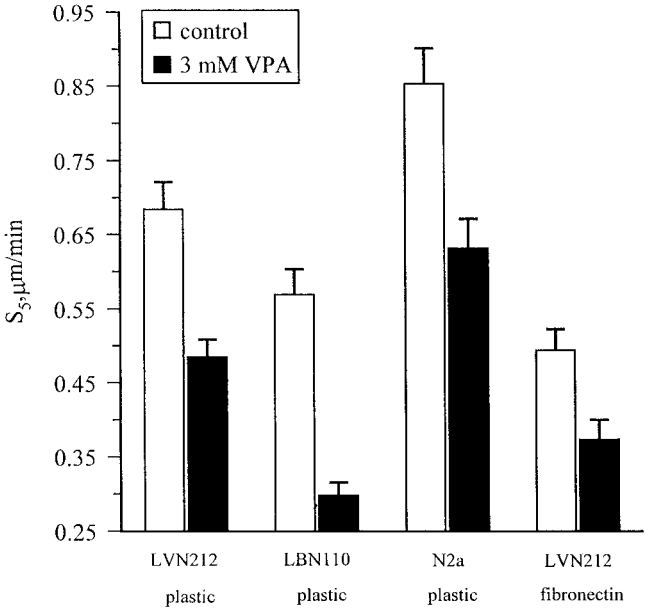


Fig. 8. Bar-diagram showing the motility of 48-h cultures treated without (open bars) or with (solid bars) 3 mM VPA. Time-lapse video-recordings were performed at 5-min intervals over a period of 20 min. S_5 was estimated by determination of the rate of centroid translocation (Materials and Methods). LVN212 is a vector transfected L-cell clone. LBN110 is a L-cell clone transfected with a human isoform of NCAM-B. N2a is a mouse neuroblastoma cell line. Cell numbers in the individual experiments range from 51–176, the mean being 108. Bars = SEM in relation to cell number. All inhibitions of cell motility caused by VPA were shown to be highly significant ($P < 0.001$) using a two-sided Student's *t*-test.

tum, being observed for cells grown on collagen I, laminin, matrigel, and fibronectin as well as on plastic [Berezin et al., 1996]. In order to demonstrate whether a similar independence of the culture substratum could be observed for the effect of VPA on the mean-cell speed of L-cells, the motility of 48 h cultures of LVN212-cells seeded on 1 µg/cm^2 fibronectin and grown in the absence or presence of 3 mM VPA was investigated. Cells grown on fibronectin exhibited a significantly lower S_5 than cells grown on plastic ($P < 0.001$, Fig. 8), possibly due to increased cell-substratum interactions. Treatment of cells grown on fibronectin with VPA, however, still induced a statistically significant decrease in motility, the relative inhibition of cellular motility being of the same magnitude as when seeded on plastic (24 and 30% for fibronectin and plastic, respectively, Fig. 8). Thus, the decrease in cell motility induced by VPA seemed to be independent of the culture substratum.

VPA Causes Changes in the Organisation of the Cytoskeleton and Focal Adhesions

The inverse correlation between mean cell area and cell speed shown in Figure 7b indicates that the effect of VPA on cell morphology and motility might be interrelated. Some of the major factors affecting cell morphology as well as cell motility are the organisation and dynamics of cytoskeletal elements and focal adhesions [Ben-Ze'ev et al., 1994; Pokorná et al., 1994; Liao et al., 1995]. Thus, in order to study the effect of VPA on cell morphology in more detail, we examined the organisation of filamentous actin (F-actin), microtubules, and focal adhesions in L-cells (clone LVN101) by immunofluorescence or Texas Red-X-coupled phalloidin stainings in cells untreated or treated with 3 mM VPA.

Figure 9 shows staining of F-actin and β-tubulin. It can be seen that the large, VPA-treated cells exhibited numerous stress-fibers often with a predominant perinuclear location (Fig. 9a, c). In contrast, in the smaller cells in the control cultures F-actin was mainly present as a cortical mesh (Fig. 9b, d). Furthermore, VPA-treated cells seemed to be devoid of microspikes (Fig. 9c), which were observed in great numbers in the control cells (Fig. 9d). Conversely, both untreated and treated cells exhibited a scaffold of well-developed microtubules (Fig. 9e, f) whose organisation did not seem to be affected by VPA-treatment.

Focal adhesions were visualised using antibodies against paxillin, vinculin, focal adhesion kinase (FAK), and phosphotyrosine. From Figure 10 it appears that VPA-treatment influenced the appearance of focal adhesions. Oblong patches of paxillin (Fig. 10a), vinculin (Fig. 10c), FAK (Fig. 10e), and phosphotyrosine (Fig. 10g) immunoreactivities typical of focal adhesions were more strongly manifested in VPA-treated cells than in control cultures (Fig. 10b, d, f, h, respectively), and control cells exhibited a more diffuse staining of the cytosol than the VPA-treated cells, indicating that as a result of the VPA-treatment, a higher proportion of paxillin, vinculin, and FAK had been directed towards the focal adhesion sites. The same pattern was observed after staining for phosphotyrosine, indicating that the signal transduction from the focal adhesions might be altered by VPA-treatment.

In conclusion, VPA-treatment affected the arrangement of the actin cytoskeleton and enhanced the formation of focal adhesions. Together with the increased mean cell area of VPA-treated cells, these results indicate that the decrease in cell motility observed upon exposure to VPA might be caused by increased cell-substratum interactions. Alternatively, the effect of VPA on cell morphology and motility might be caused by alterations in the dynamics of the actin-cytoskeleton and focal adhesions.

DISCUSSION

VPA is a known human teratogen causing *spina bifida aperta* as well as numerous other malformations

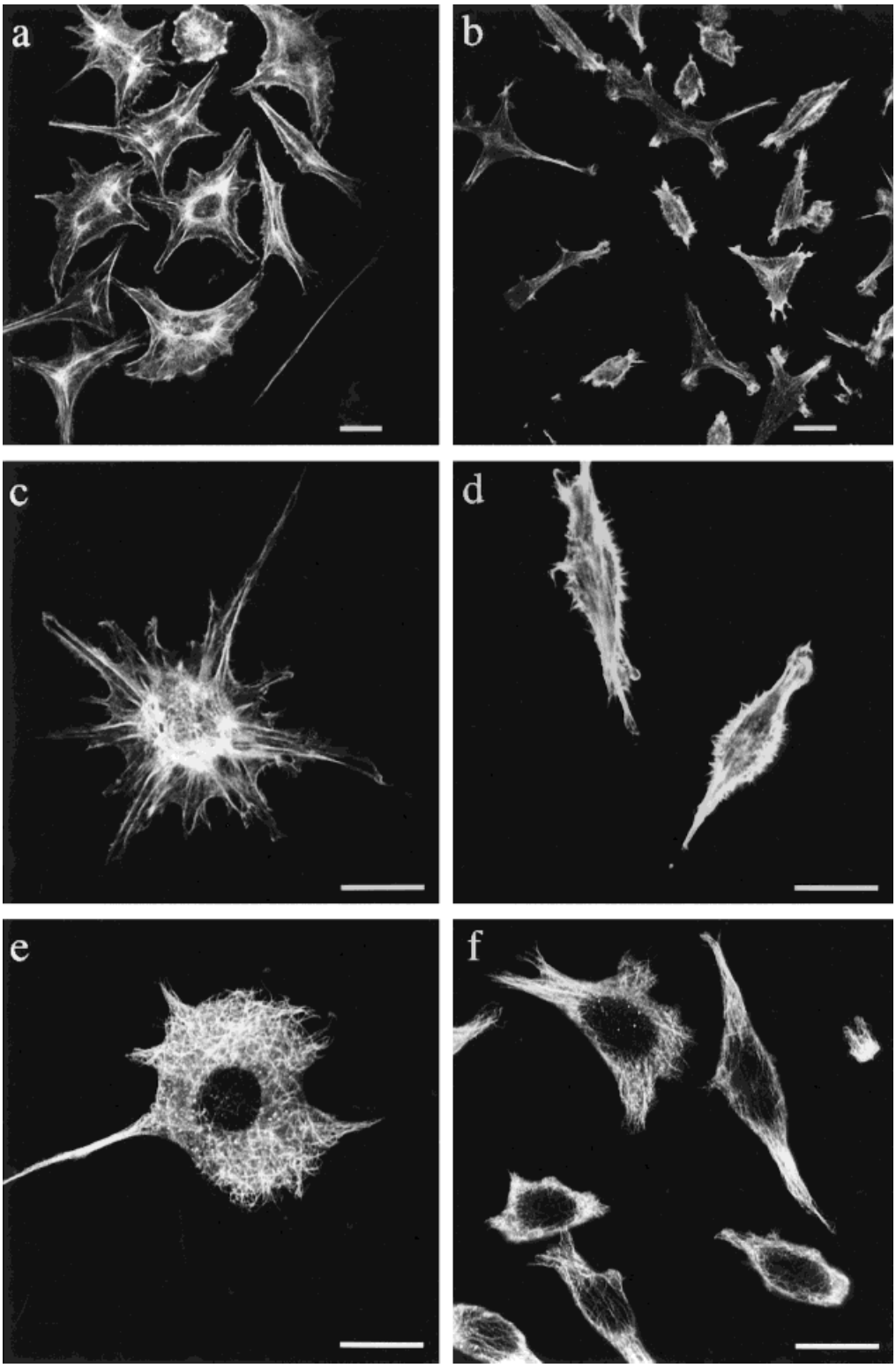


Fig. 9. Fluorescence micrographs of 48-h cultures of LVN101 cells treated without (**b, d, f**) or with 3 mM VPA (**a, c, e**). Actin filaments are stained with Texas Red X-conjugated phalloidin (a and b, low magnification; c and d, high magnification). β-tubulin is stained by indirect immunocytochemistry (e, f). All micrographs show single confocal sections taken close to the cell-substratum interface. Bar = 20 μm.

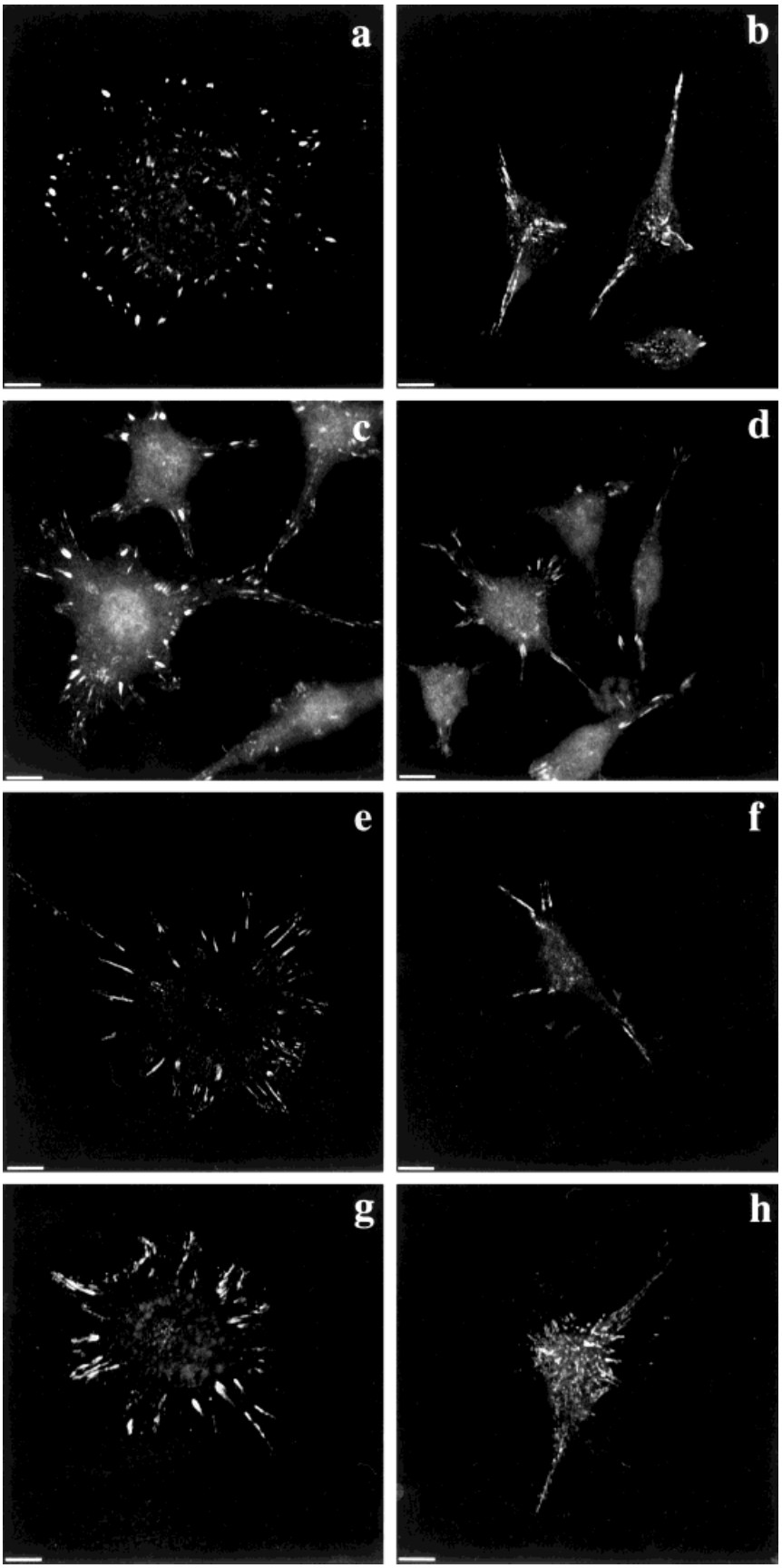


Fig. 10. Fluorescence micrographs of 48-h cultures of LVN101 cells treated without (**b, d, f, h**) or with 3 mM VPA (**a, c, e, g**). Immunostainings for paxillin (a, b), vinculin (c, d), FAK (e, f), and phosphotyrosine (g, h) are shown. All micrographs show single confocal sections taken close to the cell-substratum interface. Bar = 20 μm.

[Ardinger et al., 1988; Sharony et al., 1993]. The compound is a widely used antiepileptic drug. In 1988, one million patients were treated with it worldwide [Ehlers et al., 1992c]. Furthermore, it has been proven useful in the treatment of migraine [Jensen et al., 1994], bipolar disorder, and other psychiatric disorders [reviewed by Guay, 1995], and has been proposed as a chemotherapeutic drug in the treatment of cancer [Cinatl Jr. et al., 1996]. Thus, despite its teratogenic potential the clinical use of VPA is more likely to increase than decrease, making studies of the causes for its teratogenic effects important.

A number of VPA-analogues have been produced exhibiting no differences with regard to their sedative or anticonvulsive activities, but differing considerably in their teratogenic activities. Strict structural requirements of VPA-analogues for the expression of teratogenic effects have been demonstrated [Hauck et al., 1991; Nau et al., 1991], but so far no specific molecular target for the teratogenic activity of VPA has been found. It has been suggested that modulations of embryonic lipid-, Zn-, or folate metabolism may be involved in some aspects of VPA-induced teratogenesis [see Nau, 1994]. Thus, VPA induces a dose-dependent change of embryonic folate metabolism in vivo, and in vitro by inhibiting the activity of glutamate formyltransferase, which utilises tetrahydrofolate as a coenzyme [Wegner and Nau, 1992]. The major defect caused by VPA in foetuses, incomplete closure of the neural tube, might be explained in terms of VPA-induced changes in cell proliferation, cell adhesion, or organisation and dynamics of cytoskeletal components. The latter may result in altered cellular migration, which in turn may modify organogenesis [Ingber et al., 1994].

We have previously demonstrated that VPA and VPA-analogues were able to induce an increase of the mean cell area of cultured L-cells, and that this increase in area correlated with the teratogenic potency of the tested compounds [Berezin et al., 1996]. These observations led us to investigate the effect of VPA on the motile behaviour of L-cells.

In the present study, we show, that treatment with (*E*)-2-en-VPA, (±)-4-en-VPA, and VPA inhibited the cellular migration in a dose-dependent manner. This inhibition could be detected visually by observing the movements of cells over 10 h when presented as tracks (Fig. 1) or windroses (Fig. 2). Long-term experiments performed at a concentration of 3 mM and visualised as windroses (Fig. 2) demonstrated an inverse correlation between teratogenecity and cell displacement. However, all three investigated compounds caused a significant decrease in cellular displacement.

Long-term dose-response studies demonstrated that a reduction in the root-mean-square speed, S, and the rate of diffusion, R, was inversely correlated with the concentration of (*E*)-2-en-VPA, (±)-4-en-VPA, and VPA. However, when calculating IC_{50} for S and R, no statistically significant differences between the effects of the three compounds were demonstrated, all values being within the range of the therapeutic doses of VPA (39–154 mg/l) [Rapeport et al., 1983]. Thus, we were not able to distinguish between the effects of the non-teratogenic compound (*E*)-2-en-VPA and the two teratogenic compounds (±)-4-en-VPA and VPA on cellular motility using long-term recordings and calculations of S and R. It should be noted, however, that even though (*E*)-2-en-VPA does not induce exencephaly in vivo it has been demonstrated to cause neurobehavioral alterations in prenatally exposed rats [Fisher et al., 1994]. (*E*)-2-en-VPA, (±)-4-en-VPA, and VPA all caused a dose-dependent increase in the persistence time of direction, P (Table II). However, this increase could not be correlated to the teratogenecity of the compounds.

As an alternative to the theoretically derived root-mean-square speed, S, cellular speed was expressed as the time-interval dependent mean-cell speed, S_τ. When calculating the mean-cell speed for cells treated with (*E*)-2-en-VPA, (±)-4-en-VPA, and VPA at 15-min time-intervals, the IC_{50}-values still were not statistically significantly different from each other. However, an inverse correlation between the IC_{50}-values and the teratogenecity of the compounds was observed (see Fig. 7 and Table III), indicating that the inhibition of cell speed might indeed correlate with the teratogenic potency of the compounds. To test this in more detail, we measured the mean-cell speed of cells treated with a total of 5 different compounds with varying teratogenic potency using 5-min intervals between observations and performing recordings of cell motility for only 20 min. These experiments showed a statistically significant negative correlation between the teratogenecity of the compounds and the mean-cell speed. Also, a negative correlation between the mean cell areas and the mean-cell speeds was found. Cells exposed to the teratogenic analogues, (±)-4-en-VPA, VPA, and S-4-yn-VPA, were large and moved slowly, whereas cells in control cultures and cultures treated with the non-teratogenic compounds, R-4-yn-VPA or (*E*)-2-en-VPA, were smaller and displayed a higher mean-cell speed.

The inhibition of cellular motility was not dependent on the chosen clone of L-cells, being of the same magnitude in two other L-cell clones, LVN101 and LVN212. Furthermore, a similar inhibition was observed in the mouse neuroblastoma cell line N2a, indicating that VPA affected the motility of cell of neuroepithelial as well as fibroblastoid origin. Recently it was reported that expression of the neuronal cell adhesion molecule, NCAM (but not the expression of other cell adhesion molecules including ICAM-1, VCAM-1, ELAM-1, and LFA-3), is

upregulated in human neuroblastoma cells upon exposure to VPA [Cinatl Jr. et al., 1996]. Furthermore, we have recently shown with simple in vitro procedures that various teratogenic VPA-analogues inhibit aggregation of cerebellar neurons and formation of fasciculated neurites [Maar et al., 1997]. These results indicate that the effect of VPA may be coupled to cell adhesion phenomena, possibly through NCAM. However, introducing NCAM into L-cells did not affect the inhibitory effects of VPA (Fig. 8).

VPA and teratogenic VPA-analogues caused an increase in the mean cell area of cultured L-cells (Fig. 7a). This effect may reflect changes in cell-substratum interactions, e.g., due to alterations in the affinity or expression of integrins or due to modifications in expression or deposition of constituents of the extracellular matrix. VPA has been demonstrated to induce expression of a collagen type IV receptor in C6 glioma cells and astrocytes in primary cultures [Martin and Regan, 1988] and to cause an increase in adhesivity to naturally deposited substratum of C6 glioma cells [Maguire and Regan, 1991]. It has also been shown that VPA modifies the extracellular matrix in a three-dimensional agarose gel culture of human chondrocytes, reducing the amount of collagen type II and increasing the deposition of collagen type I [Aulthouse and Hitt, 1994]. Furthermore, VPA has been shown to prevent detachment of L-cells from naturally deposited substratum after treatment with an RGD containing peptide [Berezin et al., 1996].

When seeding L-cells on fibronectin, thereby increasing cell-substrate interactions through integrins, a significant decrease in the motility of untreated L-cells was observed when compared to L-cells seeded on plastic (Fig. 8). Thus, increasing cellular attachment seemed to mimic the effect of VPA-exposure. However, VPA-exposure of cells seeded on fibronectin caused a decrease in motility of L-cells of the same order of magnitude as when seeded on plastic (Fig. 8), indicating that altered cellular attachment may not be the only explanation for the VPA-induced inhibition of cell motility. Confocal micrographs of fluorescence stainings of microtubules, F-actin, and selected focal adhesion proteins demonstrated that VPA-exposure caused an enhanced focal adhesion formation accompanied by a rearrangement of F-actin as reflected by an increased stress-fiber formation (Figs. 9 and 10). These observations further strengthen the possibility that VPA might affect cell motility through alterations in cell-substrate interactions. Alternatively, VPA might cause alterations in the dynamics of F-actin organisation and focal adhesions. Indeed, the persistence time of cells, P, has been hypothesised to reflect dynamic cycles within the actin cytoskeleton during migration [Lauffenburger and Lindermann, 1993]. Thus, the dose-dependent increase in persistence time caused by the employed drugs may indeed reflect an interference of the compounds with cytoskeletal dynamics.

Despite the fact that cellular motility plays a key role in many biological events, little attention has been paid to the study of the effects of drugs and toxins on individual cell motility. However, whereas the cancer research field, when studying compounds suitable for chemotherapy,has so far mainly been focused on cytotoxic and cytostatic inhibitors of tumour growth, the attention towards anti-invasion and anti-metastatic drugs has recently increased [Dickson et al., 1996]. Also, the motile behaviour of cells has been proposed as a possible target for AIDS drug therapy [Soll, 1997]. Thus, a wide range of fields may benefit from simple in vitro tests for the effect of drugs, toxins, and growth factors on cell motility.

Long-term analysis has a number of disadvantages as a method for monitoring the effect of drugs on the motile behaviour of cells. First, the method is very laborious, requiring long observation times and the processing of a large amount of data. Second, the demands on the equipment used for motility recordings are considerable, including requirements for specialised hard- and software, as well as equipment ensuring stable physical conditions during recording. Even with these requirements met, the deposition of extracellular matrix, secretion of growth factors, and an increase in cell density during recording are unavoidable, making maintenance of a stable environment virtually impossible. In contrast, short-term analysis of cell motility is relatively easy to perform, since stable physical conditions are easier to maintain, and the amount of data to process is manageable. The present study demonstrates that determinations of mean-cell speed based on short-term recordings are just as sensitive a parameter as root-mean-square speed determined by long-term recordings (compare Figs. 4a and 5). The disadvantage of mean-cell speed, however, is that the actual values are dependent of the chosen time-interval [Dunn, 1983]. In order to evaluate teratogenecity of drugs, several in vitro methods are currently employed. Widely used are whole rat or mouse embryo cultures [Andrews et al., 1995; Bojic et al., 1996; Guest et al., 1994; Narotski et al., 1994], high density cultures of rodent limb bud mesenchymal cells, and other so-called micromass teratogen tests [Flint, 1993; Kochhar and Penner, 1992; Regan et al., 1990]. These methods are relatively laborious, and require the sacrifice of laboratory animals. It is, therefore, of interest to develop more simple in vitro methods for screening of teratogenic compounds. The present study has demonstrated an inverse correlation between the teratogenic potential of VPA-compounds and individual cell motility of cultured cells, although a non-teratogenic compound such as (*E*)-2-en-VPA caused a significant reduction in cell

motility. Nevertheless, cell motility studies may prove to be a useful in vitro method for the study of the teratogenic effects of the class of compounds used in the present study, branched-chain carboxylic acids, and may serve as an additional tool in the search of primary targets of the teratogenic function of VPA.

ACKNOWLEDGMENTS

The financial support of the EU-Programme on In Vitro Developmental Toxicology (contract BIO2-CT-93–0471), the EU-Programme on Neuronal Development and Regeneration (contract BIO2-CT-93–0260), the Danish Medical Research Council, the Danish Natural Science Council, the Danish Biotechnology Programme, the Danish Cancer Society, the BgVV (ZEBET, Berlin), American Biogenetic Sciences (Copiague, NY), and the John and Birthe Meyer Foundation is gratefully acknowledged.

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