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Valproate ameliorates the survival and the motor performance in a transgenic mouse model of Huntington's disease

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ABSTRACT

Huntington's disease (HD) is one of the chronic devastating neurodegenerative disorders. The pathophysiological processes clearly involve both excitotoxicity and reduced gene transcription due to the decreased level of histone acetylation, accompanied by the loss of γ-aminobutyric acidergic (GABAergic) medium-sized spiny neurons in the striatum as a pathological hallmark of HD. Thus, the antiepileptic drug valproate, which has proved GABAergic, antiexcitotoxic and histone deacetylase inhibitor effects, might be of value by exerting a beneficial neuroprotective effect. We have now tested this drug in the N171-82Q transgenic mouse model of HD, following its chronic intraperitoneal administration in a daily dose of 100 mg/kg. Valproate significantly prolonged the survival of the transgenic mice and significantly ameliorated their diminished spontaneous locomotor activity, without exerting any noteworthy side-effect on their behaviour or the striatal dopamine content at the dose administered. The beneficial effect of valproate is probably explained by its complex pharmacological activity. As several previous clinical trials carried out with valproate did not indicate any positive effect in HD, it is worth considering the design of new studies based on a well-planned treatment regime with higher dose, using valproate in monotherapy or in combination therapy with a high number of participating patients.

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1. Introduction

Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disorder manifested with cognitive, psychiatric and motor symptoms in middle-aged people. The motor symptoms include the loss of coordination of voluntary movements and the appearance of involuntary movements, such as chorea and dystonia. The disease displays a progressive nature, ending in death after a course of 15–20 years. The pathological alterations mainly affect the central nervous system and especially the striatum in which the loss of γ-aminobutyric acidergic (GABAergic) medium-sized spiny neurons (MSNs) is the most pronounced feature (reviewed by Vécsei and Beal, 1996; Walker, 2007).

HD is caused by expansion of the cytosine–adenine–guanine (CAG) repeat in the gene coding for the N-terminal region of the huntingtin protein, which leads to the formation of a polyglutamine stretch. The gene was identified in 1993 (The Huntington's Disease Collaborative Research Group, 1993), located on the short arm of chromosome 4

(4p16.3) and labelled interesting transcript 15. The physiological HD alleles comprise 6 to 35 CAGs, while alleles with 36 to 39 CAGs are characterized by reduced penetrance. Above 39 CAGs, there is obligatory disease development. The greater the expansion, the earlier the disease is manifested (reviewed by Gárdián and Vécsei, 2004).

The posttranslational acetylation of histones by histone acetyltransferases (HATs) at lysine residues results in the neutralization of those positively charged proteins, leading to a more open conformation of the DNA, allowing the binding of certain transcription factors. The histone deacetylases (HDACs) remove the acetyl groups, which results in a reduced ability to bind the transcription factors, and thus the transcription processes become considerably reduced. There is increasing evidence that an altered balance between HAT and HDAC activity may accompany the development of chronic neurodegenerative disorders, including HD (reviewed by Langley et al., 2005; Sadri-Vakili and Cha, 2006). The mutant huntingtin protein can interact with proteins which regulate transcription (reviewed by Cha, 2000; Kazantsev and Hersch, 2007), thereby deteriorating their normal function. One group of these proteins is the HATs (Igarashi et al., 2003). The inhibition of HDACs might be able to restore the altered balance described above, as the application of such substances was found to arrest the polyglutamine-dependent neurodegeneration in different models of polyglutamine disease (Steffan et al., 2001; Gárdián et al., 2005). Excitotoxicity may also play an important role in the development of HD (Coyle and Schwarcz, 1976; McGeer and McGeer,

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1976; Vécsei and Beal, 1991) in addition to or combined with proved metabolic disturbances (Klivényi et al., 2000, 2004; reviewed by Sas et al., 2007). The loss of neurons expressing N-methyl-D-aspartate (NMDA) receptors in an extremely large amount has been observed in the human striatum (Young et al., 1988) and in transgenic models. The enhanced sensitivity of the MSNs to NMDA treatment has also been described (Cepeda et al., 2001; Zeron et al., 2002; Shehadeh et al., 2006).

2-Propylpentanoic acid (valproate) is a widely used antiepileptic drug, although it is also applied in other indications, such as bipolar disorders, migraine and chronic neuropathic pain (reviewed by Johannessen and Johannessen, 2003). Its pharmacological action is exerted via a very complex mechanism (reviewed by Löscher, 1999; Kostrouchová et al., 2007). It can increase brain GABA levels (Taberner et al., 1980) through the reduction of GABA-transaminase, increasing the glutamate decarboxylase activity (Löscher, 1981a,b), and it can potentiate the release of GABA (Gram et al., 1988). Furthermore, valproate can suppress NMDA-evoked transient depolarizations (Zeise et al., 1991), and it can therefore exert a dose-dependent neuroprotective effect against excitotoxicity (Hashimoto et al., 2002). Besides the effects detailed above, valproate can additionally influence multiple regulatory pathways. In this manner, probably its most important effect is the inhibition of HDACs (Krämer et al., 2003); it selectively inhibits the catalytic activity of class I HDACs and induces the proteasomal degradation of class II HDACs.

The aim of our experiments was to utilize a transgenic mouse model of HD to test the applicability of valproate, which has been demonstrated to be protective in the malonate model of HD (Morland et al., 2004), on the basis of its proved GABAergic, antiexcitotoxic and HDAC inhibitor (HDACi) effects. Moreover, we measured the striatal levels of dopamine and two of its metabolites after chronic valproate administration, because valproate can exert effects on the brain dopamine concentrations (Vriend and Alexiuk, 1996) which might influence the motor performance of mice.

2. Materials and methods

2.1. Material

Sodium valproate was obtained in the form of powder for injections (Depakine; Sanofi Winthrop Industrie, Gentilly, France).

2.2. Animals

The transgenic N171-82Q male mice were originally purchased from the Jackson Laboratories (Bar Harbor, ME, USA), and were maintained on the B6C3F1 background in our laboratory. The offspring were genotyped by using a PCR assay on the tail DNA. The mice were housed in transgenic cages (at most 5 per cage), with free access to food and water, under standard conditions. Male and female transgenic mice and littermates were distributed equally between the experimental groups. All animal experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved in advance by the local animal care committee.

2.3. Treatments

The mice received intraperitoneal injections of sodium valproate (100 mg/kg/day, in a volume of 5 ml/kg, dissolved in saline) or the vehicle (in a volume of 5 ml/kg) at the same time each day on 5 days per week from 7 weeks of age.

2.4. Survival

The transgenic mice were treated with sodium valproate ($n = 8$) or vehicle ($n = 8$), according to the regime detailed above, until death occurred. The littermates treated with vehicle served as negative controls ($n = 7$) in order to be able to exclude other possible causes of death than those due to the presence of the transgene.

2.5. Open-field test

The treatment regime was the same as in the examination of survival. We examined the spontaneous locomotor activity and the exploration activity of the mice once a week, 2 h after the treatment, on the same day each week. The tests were performed at the same time of day so as to avoid possible changes due to the diurnal rhythm. Each mouse was placed at the centre of a box ($48 \times 48 \times 40$ cm) and its behaviour during 5 min was recorded with the aid of Conducta 1.0 software (Experimetria Ltd., Budapest, Hungary). The ambulation distance, the mean velocity, the local time, and the number of rearings were evaluated. To test the possible effects of valproate on the spontaneous locomotor activity, wild-type B6C3F1 mice were divided into two groups ($n = 8$ in each group), which received valproate or vehicle for 9 weeks according to the treatment regime detailed above. Open-field tests were performed once a week, similarly as in the former experiment.

2.6. HPLC measurements

In week 9 of treatment, the valproate-treated and control wild-type mice were decapitated and the brains were rapidly removed and placed on an ice-cooled plate for dissection of the striatum. After dissection, the samples were stored at -70 °C until measurements. The striata were weighed and were then manually homogenized in an ice-cooled solution (250 μl) comprising perchloric acid (7.2 μl; 70% w/w), sodium metabisulfite (1 μl; 0.1 M), disodium ethylenediaminetetraacetate (1.25 μl; 0.1 M) and distilled water (240.55 μl) for 1 min in a homogenization tube. The content of the homogenization tube was quantitatively washed into a test tube with the used solution to give a final volume of 2 ml, which was centrifuged at 4000 g for 30 min at 4 °C. From the supernatant, 250 μl was transferred to a test vial, and dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were analysed by reversed-phase chromatography, using an Agilent 1100 high performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA, USA) combined with a Model 105 electrochemical detector (Precision Instruments, Marseille, France) under isocratic conditions. In brief, the working potential of the detector was set at $+750$ mV, using a glassy carbon electrode and an Ag/AgCl reference electrode. The mobile phase containing sodium dihydrogenphosphate (75 mM), sodium octylsulfate (2.8 mM) and disodium ethylenediaminetetraacetate (50 μM) was supplemented with acetonitrile (10% v/v) and the pH was adjusted to 2.8 with phosphoric acid (85% w/w). The mobile phase was delivered at a rate of 1 ml/min at 40 °C onto the reversed-phase column (HR-80 C18, 80×4.6 mm, 3 μm particle size; ESA Biosciences, Chelmsford, MA, USA) after passage through a pre-column (Hypersil ODS, 20×2.1 mm, 5 μm particle size; Agilent Technologies, Santa Clara, CA, USA). Ten-microlitre aliquots were injected by the autosampler with the cooling module set at 4 °C.

The signals captured by the Model 105 electrochemical detector were converted by an Agilent 35900E dual-channel interface and the chromatograms were evaluated by ChemStation Rev.1.10.02 software (Agilent Technologies, Santa Clara, CA, USA).

2.7. Statistical analysis

Survival data were analysed by using Kaplan–Meier survival curves and the Mantel–Cox log rank test. For the statistical evaluation of the data in the behaviour tests and biochemical analyses, we used the independent t test when 2 groups were involved, and analysis of variance followed by Fisher's LSD post hoc test (StatView 4.53 for Windows, Abacus Concepts Inc., Berkeley, CA, USA) when there were more than 2 groups.

3. Results

3.1. Survival

Valproate administered intraperitoneally in a dose of 100 mg/kg on 5 days per week from the age of 7 weeks significantly increased the mean survival time of the transgenic mice from 109.8 ± 9.7 days to 144.3 ± 7.9 days, i.e. by 31.4% ($p < 0.05$; Fig. 1).

3.2. Behavioural tests

The monitored parameters indicated that mild improvements were already observed in the valproate-treated group at 9 weeks of age (week 3 of treatment), and the changes became significant by 12 weeks of age (week 6 of treatment). Thus, as concerns the spontaneous locomotor activity at 12 weeks of age (week 6 of treatment), valproate significantly increased the ambulation distance ($p < 0.001$) [$F(2,20) = 7.87$, $p < 0.01$] and the mean velocity during ambulation ($p < 0.001$) [$F(2,20) = 12.29$, $p < 0.001$], and significantly decreased the local time ($p < 0.001$) [$F(2,20) = 10.55$, $p < 0.001$] of the transgenic mice as compared with those of the untreated transgenic animals (Fig. 2a, c, e). Furthermore, from the aspect of the exploratory behaviour, the treatment significantly increased the number of rearings ($p < 0.05$) [$F(2,20) = 6.91$, $p < 0.01$] which was reduced in the transgenic animals ($p < 0.01$; Fig. 2g). The increase in spontaneous locomotor activity was also significant as compared with the wild-type mice both in the ambulation distance ($p < 0.05$) and in mean velocity during ambulation ($p < 0.01$). At 15 weeks of age (week 9 of treatment), the differences in ambulation distance and mean velocity during ambulation were not significant, due to an increase in the standard deviation. However, the significant differences in local time ($p < 0.001$) [$F(2,20) = 17.78$, $p < 0.001$] and rearing count ($p < 0.05$) [$F(2,20) = 11.41$, $p < 0.001$] still remained between the valproate-treated and untreated transgenic animals. In the applied dose, valproate did not influence either the spontaneous locomotor activity or the exploratory behaviour of the wild-type mice (Fig. 2b, d, f, h).

3.3. HPLC measurements

Valproate, administered intraperitoneally in a dose of 100 mg/kg on 5 days per week for 9 weeks did not induce significant changes in the striatal dopamine (Fig. 3a), DOPAC (Fig. 3b) or HVA (Fig. 3c) levels.

4. Discussion

Although the prevalence of HD is rather low (~5/100 000; Folstein et al., 1987; Evers-Kiebooms et al., 2002), the disease displays a pro-

gressive nature and certainly ends in death. It is known that a definite single mutation leads to the development of the disease (the best example of trinucleotide repeat expansion disorders), but many questions remain to be answered. The pathomechanism must be thoroughly explored, and effective new drugs with higher therapeutic value must be developed. At present, only symptomatic therapy is available, with neuroleptics, dopamine-depleting agents, antidepressants, antiglutamatergic drugs, GABA agonists, antiepileptics, acetylcholinesterase inhibitors and the botulinum toxin (reviewed by Adam and Jankovic, 2008). A number of agents have proved protective in transgenic models, and have recently been tested in early-phase clinical trials. Depending on the molecular mechanism of their effect, the aims mostly involve the inhibition of transcription and histone deacetylation, the achievement of marked antioxidant and antiexcitotoxic effects, improvement of the mitochondrial function and the prevention of an energy impairment (reviewed by Hersch and Ferrante, 2004). The therapeutic potency of HDACi molecules is well supported by the fact that suberoylanilide hydroxamic acid with HDACi activity markedly ameliorated the motor performance in the R6/2 transgenic mouse model of HD (Hockley et al., 2003). Some other HDACi molecules, such as sodium butyrate (Ferrante et al., 2003), phenylbutyrate (Gárdián et al., 2005) and pimelic diphenylamide HDACi (HDACi 4b; Thomas et al., 2008), have also proved protective in transgenic mouse models of HD.

In our experiments, we used the N171-82Q transgenic mouse model of HD, in which the developing motor symptoms (Klivényi et al., 2006) are clearly observed. Valproate increased the survival time of the transgenic mice significantly (by 31.4%). This elevation is noteworthy, as phenylbutyrate with proved HDACi activity lengthened the survival by 23% (Gárdián et al., 2005). Although a reduced number of animals has been included in our study ($n = 8$ in both the valproate-treated and the untreated transgenic groups) compared with the experiment with phenybutyrate ($n = 24$ in the treated and $n = 12$ in the untreated transgenic group), the differences caused by valproate treatment are clearly significant and correspond well to our behaviour data and the characteristic of the survival curves can be well studied. Nevertheless the experimental findings of these studies can only be cautiously judged due to the differences in the number of animals. As regards the spontaneous locomotor activity observed in the open-field test, valproate exerted a significant protective effect at 12 weeks of age (week 6 of treatment), and as regards the ambulation distance and mean velocity during ambulation, the treatment caused significant increases in the transgenic animals as compared with the untreated wild-type mice, too. It is worth noting here that there was no detectable side-effect of the treatment in the wild-type mice as concerns the spontaneous locomotor activity; only the number of rearings was moderately elevated. Thus, as we did not observe a similar difference between the treated and the untreated wild-type mice, the considerable increase in locomotion mentioned above is probably due to the combined effect resulting from the treatment and the presence of the transgene. Accordingly, the phenomenon can be explained in that there are regional differences in the effect of valproate on the GABA levels in certain brain areas. For example, there is a considerable elevation in midbrain regions such as the substantia nigra (Iadarola et al., 1979; Löscher and Vetter, 1985), the reticular part of which is an important part of both direct and indirect pathways of the basal ganglia circuitry. In HD, the function of the indirect pathway deteriorates first, while the direct pathway remains intact in the early stages (reviewed by Ramaswamy et al., 2007), resulting in hyperkinetic movements in humans. The treatment probably shifts the function of the direct pathway by elevating the GABA level in the substantia nigra pars reticulata with decreased inhibition of the thalamus, resulting in increased locomotion. It is also worth mentioning that chronic valproate treatment did not influence the striatal levels of dopamine and its metabolites, which would also have influenced the motor performance. Preliminary examinations on brain pathology by means of cresyl violet staining and anti-huntingtin
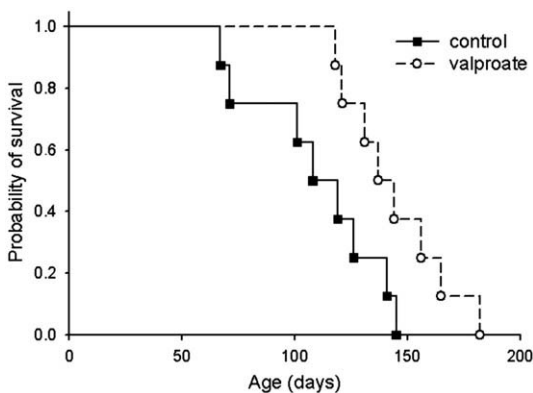

Fig. 1. Kaplan–Meier survival curves of control and valproate-treated N171-82Q transgenic mice. The treatment with valproate significantly increased the survival duration relative to the controls. ($n = 8$; $p < 0.05$, Mantel–Cox log rank test).
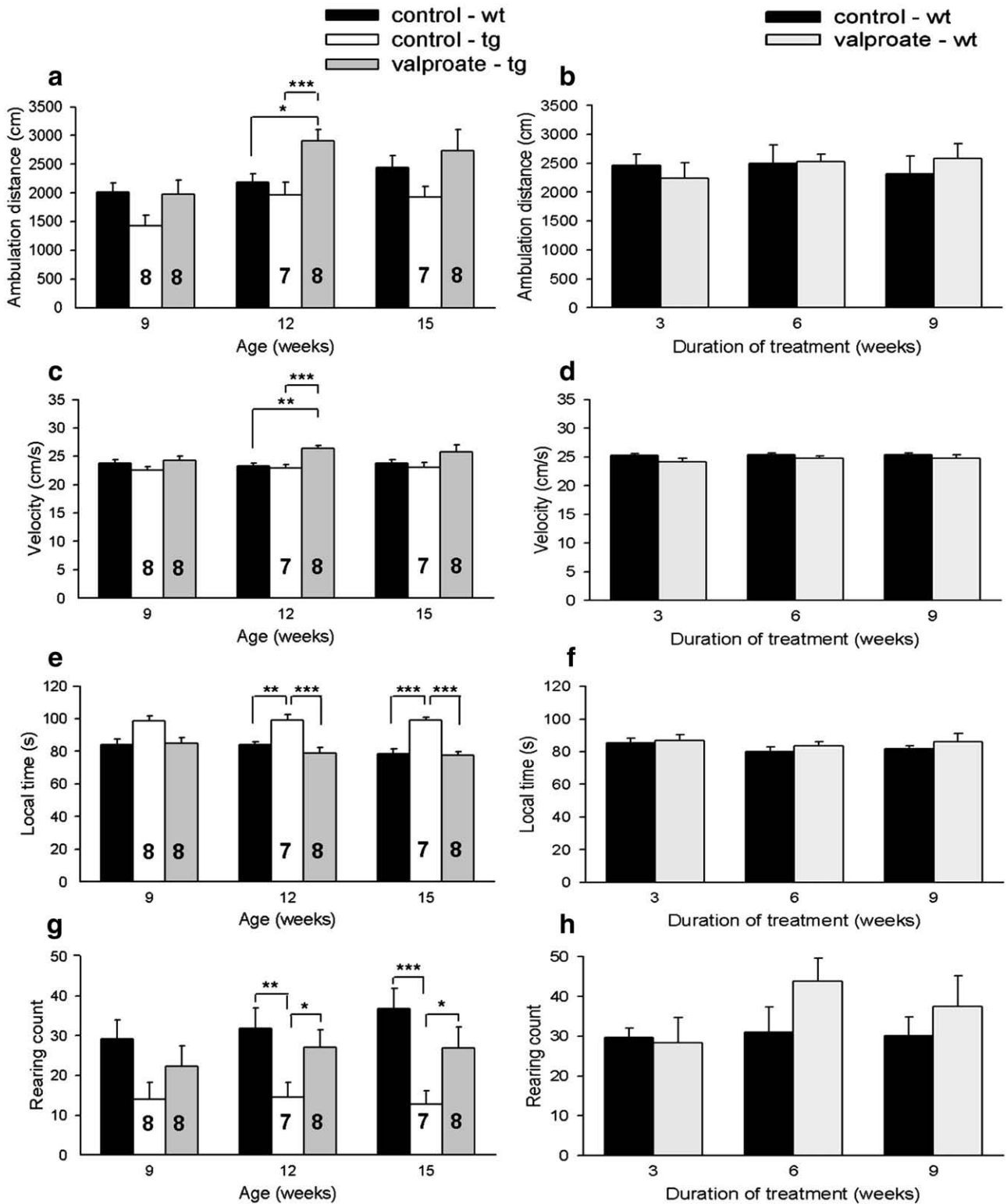
Fig. 2. The effects of valproate treatment on the spontaneous locomotor activity and exploratory behaviour in the open-field test. Valproate significantly increased the ambulation distance (a; $p < 0.001$), the mean velocity (c; $p < 0.001$) and the number of rearings (g; $p < 0.05$), and significantly decreased the local time (e; $p < 0.001$) of the transgenic mice at the age of 12 weeks (week 6 of treatment) as compared with the transgenic controls from which the differences in local time ($p < 0.001$) and in the number of rearings ($p < 0.05$) still remained significant at the age of 15 weeks (week 9 of treatment). As compared with the wild-type mice, the group means for the valproate-treated animals were also significantly higher as concerns the ambulation distance ($p < 0.05$) and velocity ($p < 0.01$); nevertheless, the valproate treatment of the wild-type mice did not lead to any significant difference as compared with the control group (b, d, f, h). ($n = 8$ wild-type mice, the numbers of mice in the transgenic groups are presented on the bar charts; tg: transgenic; wt: wild-type; data are means + S.E.M.; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

immunohistochemistry indicate that the cellular atrophy was present in treated animals, but its extent was lower than that of untreated transgenic mice (data not shown). These data are consistent with the findings in studies with other HDACis, but valproate does not seem to exert such pronounced effects as those substances. Contrary to the previous studies with HDACi molecules, where the huntingtin
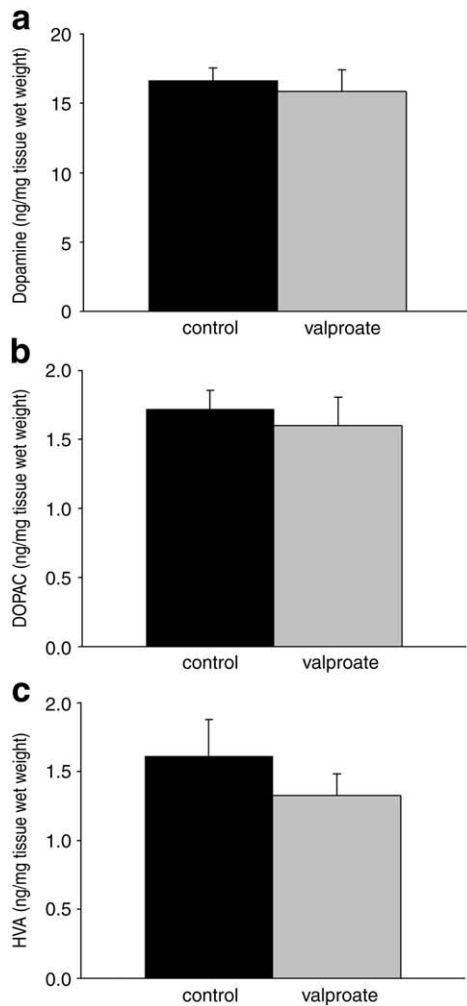
Fig. 3. Striatal dopamine, DOPAC and HVA concentrations of valproate-treated wild-type mice after 9 weeks of treatment. Valproate treatment did not significantly influence the striatal level of dopamine (a), or DOPAC (b), or HVA (c) as compared with the untreated wild-type mice. ($n = 8$; data are means + S.E.M.).

aggregate deposition was not altered, our provisory data suggest that valproate treatment diminished the striatal anti-huntingtin immuno-reactivity (examined with EM48 antibody). This phenomenon might be explained in that the pharmacological activity of valproate is more complex than its effects can be interpreted solely upon its HDACi activity.

In the 1970s, several clinical trials were carried out with valproate, usually with the aim amelioration of the choreiform movements, but without any positive effect (Bachman et al., 1977; Pearce et al., 1977; Symington et al., 1978). In the present decade, two studies have revealed that valproate is beneficial in monotherapy in the treatment of myoclonus in HD (Saft et al., 2006) and in combination therapy with olanzapine in the treatment of psychosis and movement disorders related to HD (Grove et al., 2000).

In summary, the beneficial effects of valproate in our experiments are probably due to its complex activity, i.e. the increases in GABAergic function, and antiexcitotoxic and HDACi effects. However, we should be cautious with the interpretation of the results, especially when the findings of previous clinical trials are considered. The treatment with valproate merely delayed the onset of the symptoms, and did not alter the characteristics of the survival curve; its effect therefore seems to be mainly symptomatic, which is supported by the preliminary findings on brain pathology. Furthermore, we began our experiments before the onset of motor symptoms, whereas the earlier studies were carried out on patients with manifest clinical symptoms of HD. Nevertheless, it

is worth considering the design of further preclinical studies with valproate and upon reinforcing results the set up of new clinical trials based on a well-planned treatment regime, using valproate in monotherapy or in combination therapy with a high number of participating patients.

Acknowledgments

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