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Maternal exposure to the antiepileptic drug vigabatrin affects postnatal development in the rat

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Abstract The objective was to investigate, in the rat, the effects of maternal exposure to vigabatrin (VGB) on the postnatal motor-cognitive behaviour of the offspring. We used an experimental evaluator-blind, placebo-controlled study in the rat. Ten pregnant rats were divided into five groups and treated with different doses of VGB (250, 500, 750, 1000 mg/kg/day) or placebo from gestation day (GD) 6 to GD10. After delivery, 56 pups (40 pups prenatally exposed to VGB and 16 pups exposed to placebo) were evaluated for motor-cognitive behaviour throughout postpartum day 40. At the end of testing sessions the animals were sacrificed and brain tissues processed for biochemical analysis of GABA levels. Body weight of pups and young rats whose mothers were treated with a dose of 750 mg/kg/day were significantly lower both at birth and during the whole postnatal life with respect to the control groups. Young rats of this group exhibited impaired performance in both the open-field and water maze tasks. Brain GABA contents were dramatically increased in this group of rats. No other significant nutritional, biochemical or behavioural changes were observed after treatments with doses of VGB lower than 750 mg/kg/day. The exposure to a dose of 1000 mg/kg caused abortion. Maternal exposure to VGB at relatively high doses (750 mg/kg/day) is likely to cause some important changes of the nutritional status during the pre- and postnatal life. Thus, the biochemical and cognitive abnormalities observed in this study could be related to some disturbances of brain development induced by malnutrition and/or to a disturbance of neuronal programming of the gabaergic system.

Key words Vigabatrin • Pregnancy • Antiepileptic drugs • GABA • Neuronal programming

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Introduction

Vigabatrin (4-amino-hex-5-enoic acid, vinyl-GABA; VGB) is an anticonvulsant drug and recently its use has been proposed for the treatment of cocaine addiction [1]. VGB has a highly selective mechanism of action by increasing GABA stores through the inhibition of GABA-transaminase. Its action lasts at least for 24 h and causes an increase of the whole brain GABA concentration by about 4-fold in the mouse [2]. Similar effects have been observed also in adult men by [^{1}H] nuclear magnetic resonance spec-

troscopy [3] and in children with epilepsy [4]. VGB crosses the placental filter slowly by simple diffusion through a hydrophilic pathway in women [5] and it causes a transient increase of GABA in both placenta and embryo of VGB treated mice [6]. VGB exerts well recognised therapeutic effects [7] but it causes skeletal malformations in mouse foetuses as well as defects of cranial tube [8] abnormalities of myelin formation, glial cell death [9] and apoptotic neurodegeneration on the developing rats [10].

The effect of treatment with anticonvulsant drugs on women with epilepsy, during pregnancy, is a matter of great interest both for possible malformations at birth [11] and neuropsychological disturbances throughout the postnatal life [12, 13]. Therefore, the purpose of this investigation was to analyse, in the rat, the effects of maternal exposure to VGB, during the crucial period of organogenesis, on the motor and cognitive behaviour of offspring during the postnatal life. Moreover, considering that it has been postulated that prenatal or perinatal stress and pharmacological manipulations could disturb the timetable of the expression of neurotransmitters [14], we wanted to verify if the GABA brain contents were affected after prenatal exposure to VGB.

Materials and methods

Albino female rats, CD-(SD) (Charles River Italia, Calco, Lecco, Italy) weighing 200–225 g, were used in the experiments, all performed in the Laboratory of Toxicology "F. Gorgone" (Catania, Italy), except for the biochemical analyses. Females in oestrous were mated with males and the pregnancy was confirmed by the presence of vaginal plugs or sperm in vaginal smears. Individual animals were housed in single cages, kept at room temperature (20°C) under a constant light–dark cycle (light on between 8.00 p.m. and 8.00 a.m.), with "ad libitum" access to water and standard laboratory food. All housing, care and experimental procedures conformed to the standards recommended by the Guide for the Care and Use of Laboratory Animals (Guide and Care and Use of Laboratory Animals, DHHS Publication No. NIH 85–23, 1985) and Directive 86/609 CEE (Official Journal of the European Communities N. L358/1, 18 December 1986). Eight pregnant females were divided into four groups (two rats per group) and treated with increasing doses of VGB (respectively 250, 500, 750, 1000 mg/kg/day). Doses of VGB selected in the present study were multiples of the starting dose of 250 mg/kg/day, which is recognised to control seizures induced by maximal electroshock, pentylenetetrazole and kindling [7, 15, 16]. Two other pregnant rats were treated with placebo and served as the control group. Drugs were dissolved in saline and prepared fresh daily. The dosage preparations were administered to females by "gavage" as a single daily dose, from GD6 (implantation) to GD10. Individual dose volumes were calculated daily based on the most recent body weight. After delivery, rats were allowed to litter and to rear their offspring to weaning (PPD 21). The number of pups delivered by the treated mothers is reported in Table 1. On PPD 5 the groups were reduced in size by randomly selecting four male and four female pups per litter (40 pups prenatally exposed to VGB and 16 pups exposed to placebo) that should be maintained to be tested for behaviour throughout the postnatal life until PPD40. Possible disturbances of feeding behaviour induced by a treatment were evaluated by providing controlled amounts of food pellets, and by weighing the pellets residual from the previous day each morning. A variety of standard behavioural tests, selected to specifically evaluate both motor and cognitive abilities, were administered in sequence starting from the first postnatal week up to PPD 40. Postural and locomotor behaviours during the first three postnatal weeks were tested according to a previously reported protocol [17]. Starting from the fourth postnatal week, horizontal locomotor activity in the open field was tested by allowing animals to freely move over a plexy glass flat surface (100x100 cm) divided into 16 equal 25x25 cm squares. The number of squares' lines crossed within the single 5-min testing trial gave the measure of the animal's activity level; exploratory behaviours were similarly tested by counting the number of rearings over the fence wall or nose pokings into small holes present on the floor surface. Over the last week, spatial learning abilities were evaluated in a water "T" maze (100x100 cm, 25 cm wide) by monitoring the animal's choices between entering the left or the right arms towards a hidden escape platform whose position remained unchanged. Four trials were administered every day for 5 consecutive days and responses were scored as correct or incorrect. Two different examiners, unaware of the pharmacological treatment throughout all the experimental sessions, tested the rats. Each testing session was video recorded for subsequent offline analyses. At the end of behavioural testing sessions all animals were sacrificed (PPD 40) under terminal anaesthesia and their brain tissues were removed and processed for measurement of GABA tissue levels by a sensitive amino acids analyser, using a single cation exchange column and lithium citrate buffer solution, according to the method of Stein and Moore [18] as previously reported [19].

Table 1 Number of litters, pups and their weights after maternal exposure to VGB

Drug	Doses (mg/kg/day)	Abortion (units)	Mortality (mothers)	Litters (units)	Number (units)	Weight (g)
Placebo		0	0	2	29	6.3±0.2
VGB	250	0	0	2	22	7.3±0.32
VGB	500	1	0	1	12	6.7±0.23
VGB	750	0	0	2	25	4.8±0.28*
VGB	1000	2	0	–	–	–

*$p<0.05$

Statistical analysis

Group differences in task performance or tissue neurotransmitter levels were analysed by repeated measures analysis of variance (ANOVA) or one-way ANOVA followed by *post hoc* Fisher's Protected Least Significant Difference (PLSD) test, as appropriate. All differences were considered significant at $p<0.05$.

Results

Mortality, abortions and malformations

Table 1 shows the occurrence of abortions in rats treated with 500 mg/kg/day (one rat) and 1000 mg/kg/day (two rats). The necropsy of rats not able to deliver showed a variable number of visible implantation sites between 9 and 13. No obvious malformations were observed in the surviving offspring.

Weight of pregnant rats, pups and young rats

Both during the treatment (GD6–GD10) or after the withdrawal of VGB (GD11), at all doses, no modifications in the behaviour of pregnant rats in comparison with rats treated with placebo were observed, but modifications in feeding behaviour were observed. The individual food consumption of pregnant rats was reduced markedly during the period of drug administration (GD6–GD10) as compared to GD0. In fact, whereas the two rats treated with placebo showed, respectively, a small decrease (–17%) or an increase (+42.9%) in food consumption, those treated with VGB showed a global 26–93% decrease in the various dose groups. The most dramatic decrease of food consumption was observed in the rats treated with 750 mg/kg/day (88.2–93.3%) and 1000 mg/kg/day (88.2–89.1%). After the withdrawal of VGB treatment the food consumption increased, but it did not reach the same values of the control group. The body weight gain physiologically occurring during pregnancy was arrested after the five-day treatment with VGB, at all doses. After the withdrawal of treatment, all pregnant rats increased their body weight markedly except for those treated with 750 and 1000 mg/kg/day. After the delivery, mothers treated with VGB carefully attended to pups as those of the control group, and no deaths of offspring were registered. At birth, body weight of offspring of mothers exposed to 750 mg/kg/day of VGB was significantly and markedly lower than the control group ($p<0.05$). During postnatal development, the body weight of pups and young rats prenatally exposed to the lowest doses of 250 and 500 mg/kg/day did not show significant differences with respect to that of rats exposed to the placebo. By contrast, the weight of the pups exposed to 750 mg/kg/day was significantly lower ($p<0.05$) than normal through the entire postnatal development.

Neurochemical data

No significant modifications of GABA levels for rats prenatally exposed to 250–500 mg/kg/day of VGB were observed. However, the presence of a trend toward an increase of GABA levels in the rats exposed to 500 mg/kg/day (+128%) should be noted. On the other hand, a marked increase of GABA, with respect to the values of the placebo group, was observed at PPD 40 in the rats exposed during prenatal life to 750 mg/kg/day of VGB (+391%, $p<0.05$, Fig. 1).

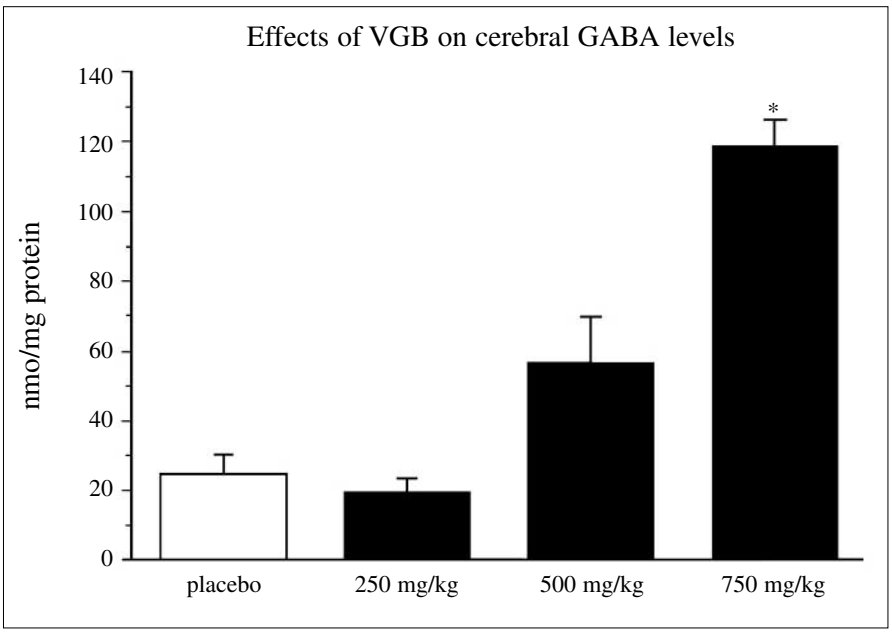

Fig. 1 Neurochemical analyses. GABA tissue levels measured in young adult rats (PPD 40) exposed during intrauterine life to increasing doses of VGB (250, 500 or 750 mg/kg/day). Asterisk indicates difference at $p<0.05$ *vs*. placebo

Behavioural evaluation

Young rats exposed during prenatal life to 250–500 mg/kg/day of VGB did not differ from those in the placebo (control) group on any of the behavioural measures analysed. By contrast, rats exposed to higher doses of VGB (750 mg/kg/day) exhibited marked changes in both motor and cognitive performances. Thus, animals in the 750 mg/kg/day group showed reduced locomotor activity as well as reduced exploratory behaviour in the open field compared to normal (Fig. 2a) (respectively –37% and –45%; in both cases $p<0.05$). When tested for spatial learning and memory in the water T-maze task, rats in the 250 and 500 mg/kg/day dose groups performed as efficiently as those exposed to placebo, whereas the rats in the 750 mg/kg/day group displayed marked acquisition deficits (Fig. 2b). In these animals, the percent of correct responses (i.e., the swim route taken towards the arm containing the hidden escape platform) during the last two days of testing, when the acquisition of the task is fairly complete, appeared significantly reduced, as compared to the animals treated with placebo ($p<0.05$). In no case were the observed impairments due to unspecific sensory-motor alterations. Animals in all treatment groups showed similar swim speeds (between 0.2 and 0.3 m/s), and performed correctly in a variety of tasks designed to test sensory-motor abilities (data not shown).

Discussion

The main findings of this study point out that prenatal exposure to VGB, at the dose of 750 mg/kg/day, during the period of organogenesis (GD6–GD10), induced firstly malnutrition in offspring and secondly some specific changes of motor and cognitive behaviour consisting of a reduction of both exploratory activity and spatial memory. By contrast, VGB at lower doses (250 or 500 mg/kg/day) did not cause any significant behavioural impairment. The highest doses of VGB (1000 mg/kg/day) caused toxic outcomes resulting in abortions. A dramatic increase of GABA cerebral levels has been observed at PPD 40 in those rats prenatally exposed to 750 mg/kg/day of VGB. Due to the short time of action of VGB on GABA-transaminase, these abnormal GABA contents cannot be caused by a direct effect of VGB on GABA system. Then, a possible disturbance of the neuronal programming of GABA neurons could be suggested.

Our study has two main limitations, the former is that in this kind of study it is very difficult to assess exactly at which concentration of VGB embryos and foetuses have been exposed during the intrauterine life and which are the blood concentrations of drug possibly affecting their postnatal development. The latter is that the experiments were not specifically designed to have a time course of the possible changes of brain GABA levels during the whole postnatal period. This issue could be addressed in a second experimental phase.

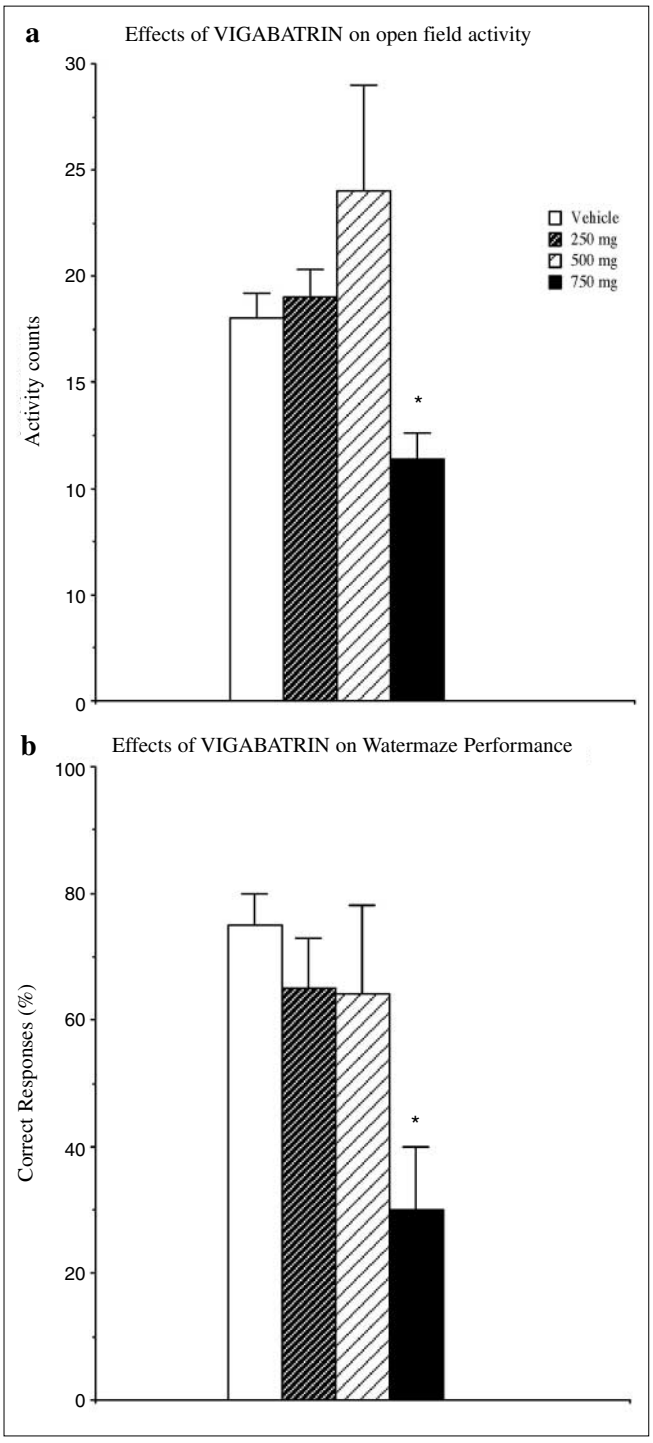

Fig. 2 Behavioural analyses. Performances in the open field and water maze tasks by rats exposed during intrauterine life to increasing doses of VGB (250, 500 or 750 mg/kg/day). Asterisks indicate difference at $p<0.05$ *vs.* placebo

Postnatal development seems to be affected differently according to the doses of VGB administered to pregnant rats. In fact, we have observed that offspring of rats exposed during pregnancy to 250 and 500 mg/kg/day of VGB showed similar body weight of control groups at birth, whereas those exposed to 750 mg/kg/day showed a lower body weight than those of the control group. This phenomenon could be dependent on intrauterine growth retardation (IUGR) that has been previously reported in mice foetuses after treatment with different doses of VGB [10] or direct effect of VGB on nutrients' transfer through placenta from mothers to embryos. In fact, Abdulrazzaq et al. [6] have shown that a single administration of 400 mg/kg of VGB, during organogenesis (GD10) significantly reduced the methionine concentration in the embryo and placenta after 9 h. According to these authors [6] a possible mechanism of this phenomenon could be dependent by a VGB-induced depletion of the enzyme and cofactors important in the metabolism of methionine.

In our experimental protocol, we have observed some disturbances of cognitive behaviour of offspring prenatally exposed to 750 mg/kg/day of VGB. These abnormalities have not been previously reported and regard two performances strictly related to the psychological status of the rat. In fact, we have observed a significant decline of both exploratory behaviour and learning of spatial memory task, suggesting the presence of some lack of interest for the environment as well as some disturbances of spatial learning. Some possible explanations – not mutually exclusive – for these long-term effects of VGB administration can be given. A first possibility is that malnutrition has caused a physical impairment leading to a lack of the motor efficiency required to perform the exploratory and the water maze tests. However, young rats exposed during prenatal life to a treatment with 750 mg/kg/day scored well in all tests of motor behaviour and also showed swimming speeds in the same range as the animals in the control group. Thus, they were smaller but physically able. A second possibility is represented by the fact that GABA has a trophic role during early brain development, namely on the hippocampus. This trophic role of GABA seems to be due to the depolarising action exerted by GABA on immature neurons by allowing the calcium to enter through both voltage-gated and NMDA (N-methyl-D-aspartate) receptor-activated channels [20]. The increase of calcium controls gene expression and neurotrophin signalling thus contributing to the construction of axonal connections [21]. GABA-mediated depolarisation induces a sort of precocious epilepsy in the hippocampus, by synchronising cell firing with large, rhythmic synaptic events and associated oscillations in intracellular Ca^{2+}. The resulting large, rhythmic synaptic events and associated oscillations in intracellular Ca^{2+} seem to provide a natural trigger for plasticity of synaptic connections and, thus, for establishing and patterning the neural network [22, 23]. Consequently, interference with GABAergic transmission during the organogenesis may affect the development of neuronal wiring and plasticity of neuronal network. Therefore it is possible to suggest that the cognitive abnormalities seen here might be the late expression of an early induced GABA dysfunction, possibly occurring in the hippocampal formation [24].

The dramatic increase of GABA brain levels observed cannot be considered a direct effect of VGB on GABA-transaminase, because the effect of this drug vanishes shortly after administration [2]. Although from our data we cannot advance any definitive explanation about this increase of brain GABA levels, it is possible that prenatal exposure to VGB, at the dose of 750 mg/kg/day, has caused an abnormal organisation of GABA circuitry that may be reflected later during the adult life. Similar dramatic delayed increases in serotonin levels have been recently detected in response to perturbed migration and differentiation of serotonergic neurons induced by administration of valproic acid on embryonic day 9 [25, 26]. Our experimental protocol does not allow the direct demonstration of a similar mechanism for GABA neurons in our preparation, but this is certainly an interesting issue that warrants further investigation.

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Sommario *Lo scopo di questa ricerca è stato di studiare, nel ratto, gli effetti della esposizione materna al vigabatrin (VGB) sullo sviluppo postnatale motorio e cognitivo delle nidiate. Il disegno è stato uno studio sperimentale "evaluator-blind" e placebo controllato. Dieci ratte gravide sono state divise in cinque gruppi e trattate con differenti dosaggi di VGB (250, 500, 750, 1000 mg/kg/giorno) o placebo dal sesto (GD6) al decimo (GD10) giorno di gestazione. Dopo il parto, 56 neonati (40 esposti nel periodo pre natale al VGB e 16 esposti al placebo) sono stati valutati per lo sviluppo motorio e cognitivo fino al quarantesimo giorno post-parto (PPD40). Alla fine delle sedute sperimentali gli animali sono stati sacrificati ed i tessuti cerebrali sono stati trattati per le analisi biochimiche dei livelli cerebrali di GABA. Il peso corporeo dei neonati e dei giovani ratti le cui madri erano state trattate con 750 mg/kg/giorno di VGB è risultato significativamente basso sia alla nascita sia durante la vita postatale rispetto ai controlli. I giovani ratti di questo gruppo hanno mostrato una inadeguata capacità sia al test "open-field" sia a quello del "water-maze". I contenuti di GABA sono aumentati in maniera drammatica in questo gruppo di ratti. Non abbiamo osservato alcuna significativa modificazione nutrizionale, biochimica o comportamentale nei ratti figli di madri esposte a dosaggi di VGB più bassi di*

750 mg/kg/giorno. L'esposizione alla dose di 1000 mg/kg/giorno ha causato aborto. L'esposizione materna a relativamente alte dosi di VGB (750 mg/kg/giorno) sembra provocare alcune importanti modificazioni sullo stato nutritivo dei figli durante la vita pre e post-natale. Di conseguenza, le anormalità biochimiche e cognitive osservate in questo studio potrebbero essere dipendenti da alcuni disturbi dello sviluppo cerebrale causati dalla malnutrizione e/o da un disturbo nella programmazione neuronale del sistema gabaergico.

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