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Activities of Proline-Specific Proteinases in the Serum and Cerebrospinal Fluid of Rats with the Fetal Valproate Syndrome

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In 60-day-old Wistar rats with fetal valproate syndrome, the brain to body weight ratio was higher by 9.4% and activity of dipeptidyl peptidase IV in the serum and cerebrospinal fluid was higher by 18.4 and 40.6%, respectively, than in healthy controls. Activity of prolylendopeptidase in the serum and cerebrospinal fluid in rats with the fetal valproate syndrome did not differ from the control.

Key Words: *fetal valproate syndrome; Wistar rats; dipeptidyl peptidase IV; prolylendopeptidase*

The fetal valproate syndrome (FVS) induced in Wistar rats by prenatal administration of valproic acid in a high dose serves as one of the most adequate models reproducing phenotypes with behavioral characteristics resembling autism spectrum disorders (ASD) in humans [8,10]. The etiology of ASD is obscure in the majority of cases [5]; several theories were proposed for explanation of the disease development [3]. According to the exorphin theory, high content of exorphins (alimentary opioid peptides casomorphin, gluten exorphin, and gliadorphin) in the CNS of patients with ASD leads to the formation of autism symptoms [15]. According to this theory, high content of exorphins in the CNS of infants with ASD results from low level of blood dipeptidyl peptidase IV (EC 3.4.14.5, CD26, DPP-4) that cleaves exorphins [7]. In our model, ASD was modeled by using sodium valproate (SV). This drug inhibits another proline-specific proteinase, prolyl endopeptidase (EC 3.4.21.26; PEP), in patients with manias [13] and in experimental mice [12].

We studied activities of proline-specific proteinases DPP-4 and PEP in the serum and cerebrospinal fluid (CSF) of mice with ASD. As the brain of autistic children is characteristically larger than normally [9,11,14], we compared brain-to-body weight ratios in groups of animas with experimental pathology and without it.

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MATERIALS AND METHODS

The study was carried out on male Wistar rats, offspring of female and male Wistar rats purchased from Stolbovaya Breeding Center (Moscow region). The rats were kept under standard vivarium conditions with free access to water and fodder at 12/12 h day/night schedule. The animals were maintained in accordance with Sanitary Rules (SP 2.2.1.3218-14, issued August 29, 2014) for organization, equipping, and maintenance of experimental biological clinics (vivaria); Order No. 51. The work was organized and carried out in accordance with Order No. 199n of the Ministry of Health of the Russian Federation (Concerning Approval of Due Rules of Laboratory Practice, April 1, 2016) and European Convention for Protection of Vertebrates used for Experimental or Other Scientific Purposes (Strasbourg, 1986). The experiment was approved by the Ethic Committee of V. V. Zakusov Research Institute of Pharmacology.

FVS was induced by intraperitoneal injection of 500 mg/kg SV (Sigma-Aldrich) to females on day 13 of gestation. Controls were intraperitoneally injected with aqua pro injection. The males were selected from the resultant litters for further experiments. On day 60

of life, the rats were narcotized with sodium ethaminal (40 mg/kg intraperitoneally) and CSF was collected from the cisterna magna (pontocerebellar cistern). The blood was collected after decapitation and the serum was separated by centrifugation. The brain-to-body weight ratio was calculated.

In rat serum and CSF, activities of peptide metabolism enzymes DPP-4 and PEP were measured fluorometrically [1] using peptides Z-Ala-Pro-MCA and Gly-Pro-MCA as the substrates. The samples were incubated for 15 min (serum) and 4 h (CSF) for DPP-4 measurements and 25 min and 4 h, respectively, for PEP measurements.

SV solution in water or reference PEP inhibitor Z-Met-PrdN [2,4] in DMSO were added *in vitro* to samples for measuring enzyme activities. For *ex vivo* experiments, Wistar rats were intraperitoneally injected with SV (500 mg/kg), controls intraperitoneally received an equivalent volume of water for injections. The animals were decapitated 1 h after SV injection, the brain was removed, and 5% homogenate was prepared. PEP activity in the brain homogenate and serum was measured as described previously.

The results were statistically processed using Statistica 10.0 software. The normality of distribution was verified by the Shapiro—Wilk test followed by evaluation of equality of dispersions by Levene test. In case of normal distribution in the groups and equality of dispersions between the groups, the data were processed by Newman—Keuls test. In case of inequality of dispersions, the data were processed using Mann—Whitney test. The results are presented as the mean±error in the mean (*M*±*SEM*). The differences between the groups are significant at $p<0.05$.

RESULTS

The weight of the brain increased in rats with FVS, which was seen from a significant increase in brain-to-body weight ratio (by 9.4%; $p=0.036$, Newman—Keuls test) in comparison with the control. In the control group, the brain-to-body weight ratio was 0.415±0.016 *vs.* 0.454±0.009 in the FVS group.

In animals with experimental ASD, similarly as in humans with this pathology, DPP-4 activity differed from the normal, but the vector of these shifts in experimental ASD was opposite to changes observed in the patients. A pilot study [6] has demonstrated that serum concentrations of DPP-4 in autistic children were significantly below the normal and that the concentrations of DPP-4 in children with severe forms of the disease were significantly lower than in children with mild to moderate ASD symptoms.

In our study, 60-day-old rats with FVS had increased activity of DPP-4. Serum DPP-4 activity surpassed the control by 18.4%, while DPP-4 activity in CSF increased even more dramatically and surpassed the control by 40.6%. By contrast, PEP activities in the serum and CSF were virtually the same in control rats and rats with FVS (Table 1).

Increased DPP-4 activity in CSF and serum of rats with FVS can be presumably explained by a compensatory reaction to prenatal administration of SV in a high dose suppressing PEP activity [12,13]. In order to verify this hypothesis, we carried out additional experiments to evaluate the relationship between SV and PEP activity *in vitro* and *ex vivo*.

In vitro experiments revealed no effect of SV on PEP activity: enzyme activity after SV injection did not differ from the control (in contrast to administration of reference PEP inhibitor Z-Met-PrdN [2,4]) (Fig. 1).

Ex vivo experiment showed that PEP activities in the serum and brain homogenates from rats treated with SV did not differ from those in control animals. Hence, our experiments showed no inhibitory effect of SV on PEP activity observed in patients with manias [13] and in experimental mice [12]. This could be explained by the pathogenesis of manic disorders in humans and by species-specific differences. In addition, differences effects of SV on PAP activity could be explained by the use of different substrates: in our study it was Z-Ala-Pro-MCA with almost 10-fold higher

TABLE 1. Activities of DPP-4 and PEP (nmol/ml/min) in the Serum and CSF of Rats with FVS (*M*±*SEM*)

Group	DPP-4		PEP	
	blood serum	CSF	blood serum	CSF
Control	24.45±0.87 (*n*=9)	1.65±0.07 (*n*=9)	1.08±0.08 (*n*=10)	0.34±0.03 (*n*=9)
FVS	28.95±1.01* (*n*=15)	2.32±0.17^{+} (*n*=9)	1.07±0.11 (*n*=15)	0.36±0.04 (*n*=9)

Note. *$p=0.006$ in comparison with the control (Mann—Whitney test); $^{+}p=0.005$ in comparison with the control (Newman—Keuls test).

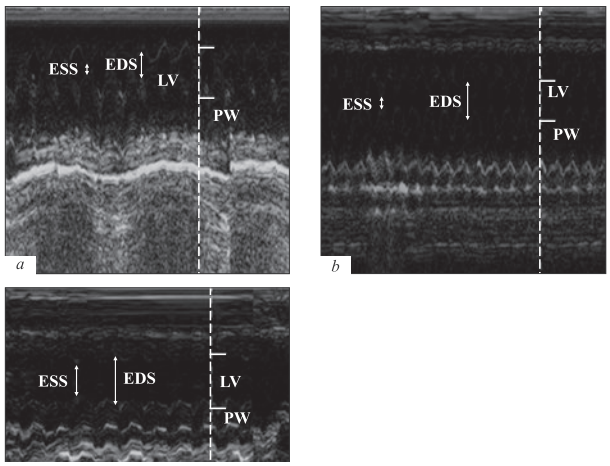

Fig. 1. Effects of SV and PEP inhibitor Z-Met-PrdN on PEP activity.

affinity for the enzyme than Suc-Gly-Pro-MCA used in other studies [12,13]. According to our data, the Michaelis constant was 0.3 mM for Suc-Gly-Pro-MCA and 0.05 mM for Z-Ala-Pro-MCA.

Additional experiments did not confirm the hypothesis on the relationship between the compensatory increase of DPP-4 activity in response to prenatal high-dose SV and its effect on PEP activity. Presumably, SV, injected to pregnant females, triggered modification of the enzymatic system activity in the progeny, the mechanism of these changes not related to direct modulation of the system. This problem deserves further studies.

Hence, increased brain-to-body weight ratio in 60-day-old rats with experimental FVS in comparison with control animals is an important characteristic of the FVS model. The detected morphological feature indicates that the chosen model allows adequate reproduction of ASD, as, according to some authors [9,11,14], the brain of autistic infants is larger than that of normal infants. Studies of the activities of proline-specific proteinases showed that changes in DPP-4 activity typical of ASD were not observed in rats with FVS. Moreover, activity of the enzyme increased in the serum and CSF of 60-day-old rats with FVS, which could be regarded as a characteristic feature of the chosen experimental model of ASD. Further studies of the time course of proline-specific proteinase activities in aging animals should be carried out.

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