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THE EFFECT OF 6-HYDROXYDOPAMINE ON EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN THE RAT

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The possible role of catecholamines in the development, course and intensity of autoimmune allergic encephalomyelitis was investigated. To this aim, catecholamines were first depleted by pretreatment of the rats with 6-hydroxydopamine (6-OHDA), a substance known to destroy adrenergic structures. 6-OHDA was applied 5 times intraperitoneally and/or intracerebroventricularly. After pretreatment with 6-OHDA the animals were immunized with an emulsion of guinea-pig spinal cord.

The results indicate that pretreatment with 6-OHDA produces a suppressive effect on the development, intensity and duration of autoimmune encephalomyelitis.

Key words: 6-Hydroxydopamine - Chemical sympathectomy - Immune response - Experimental allergic encephalomyelitis

INTRODUCTION

The immune system has been postulated as a microenvironment in which the immune response is closely correlated with products of the endocrine and nervous systems (Janković, 1979; Janković and Isaković, 1973). The neuroendocrine system has been already shown to play a significant role in the immune response (Ader, 1981; Janković et al., 1987).

Thus, selective lesion of adrenergic neurons by 6-OHDA has been shown to depress the primary immune response (Roszman et al., 1985), as well as the Arthus phenomenon, delayed skin hypersensitivity reactions (Ćupić et al., 1994 in press) and the plaque-forming cell response (Ćupić et al., 1994a in press).

Experimental autoimmune encephalomyelitis is known to include significant changes in the central nervous system. It was there fore of interest to investigate the possible role of the catecholaminergic system in the development, severity and duration of experimental allergic encephalomyelitis in rats.

MATERIALS AND METHODS

The experiment included 8-week-old rats with body mass from 200 to 250 g. A group of these animals was implanted with a polyethylene cannula intracerebroventricularly (i.c.v.), according to the method of Feldberg and Sherwood (1953).

6-OHDA was injected intraperitoneally (i.p.) (20 mg/kg b.m.) or i.c.v. (0.6 mg/kg b.m. in a volume of 10 μl). The control group of animals was injected with physiological saline in the same way and in the same volume. 6-OHDA was injected 5 times, once every second day starting on the 8th day before immunization up to the very day of immunization (day O.).

The animals were immunized with emulsified guinea-pig spinal cord and complete Freund's adjuvant. The animals were injected intradermally (i.d.) into the hind paw with 0.1 ml of emulsion containing 20 mg of spinal cord. Immediately after injection of antigen, 0.5 ml of Bordetella pertussis vaccine was injected into the dorsal part of the same hind paw. This type of treatment served as a complementary adjuvant. The intensity of the disease was evaluated according to the severity of the neurological signs: O-absence of any neurological signs, + - loose tail, ++ - paresis of the hind extremities, +++ - paralysis of the hind extremities, ++++ - quadriplegia. On the 15th day after immunization, all the animals were sacrificed, and the brain and spinal cord were dissected for histological analysis. The intensity of histological lesions was evaluated also from O to ++++, depending on the number of perivascular mononuclear infiltrates which are characteristic of experimental allergic encephalomyelitis. Statistical evaluation of the results was done using Student's T-test.

RESULTS

The effect of multiple application of 6-OHDA on the general characteristics of experimental allergic encephalomyelitis (EAE) – In animals pretreated with 6-OHDA a tendency for a decrease in the severity of the general characteristics of EAE in rats was observed. Thus, the incidence of the disease was slightly lower, the begining of the disease was slightly delayed and the duration of the disease was shortened. However statistical significance was reached only in the group of animals pretreated with 6-OHDA both i.c.v. and i.p. in which the duration of EAE was significantly shortened. There was no statistically significant difference in the mortality rate between the animals treated with 6-OHDA and controls treated with physiological saline. These results are shown in Table 1.

The effect of multiple application of 6-OHDA on the intensity of clinical signs of EAE - The mean score indicating the intensity of clinical signs of EAE in rats was significantly decreased only in animals pretreated with 6-OHDA both i.c.v. and i.p. (Table 2.) This was not observed in the groups of animals treated either i. p. or only i.c.v.

Table 1. The effect of multiple application of 6-OHDA on general characteristics of experimental allergic encephalomyelitis (EAE) in rats. There were 10 animals in each group. The values represent the mean± S.D.

Group Treatment	Way of application	Incidence of the disease (%)	Days to begining of the disease	Duration of the disease (in days)	Mortality (%)
Experimental					
6-OHDA	i.p	80	10.3±3.0	4.7±2.1	10
6-OHDA	i.c.v.	90	10.0±1.8	4.0±1.3	10
6-OHDA	i.c.v. + i.p	90	10.4±4.0	3.0±2.1*	10
Controls					
Physio-logical saline	i.p.	100	9.6±0.9	4.7±0.9	10
—	i.c.v.	100	9.0±3.7	4.5±2.4	20

* P<0.05 in comparison with physiological saline group treated i.c.v.

Table 2. The effect of multiple application of 6-OHDA on the intensity of clinical signs of experimental allergic encephalomyelitis (EAE) in rats. There were 10 animals in each group. The values represent the mean± S.D.

Group	Treatment	Way of application	Number of animals with clinical signs					Mean score (M±SD)
			0	+	+	+++	++++	
Experimental								
	6-OHDA	i.p	2	0	1	4	3	2.6±1.5
	6-OHDA	i.c.v.	1	1	3	3	2	2.4±1.2
	6-OHDA	i.c.v. + i.p	1	3	3	1	2	2.0±1.3*
Controls								
	Physio-logical saline	i.p.	0	1	3	4	2	2.7±0.9
	—	i.c.v.	0	1	2	5	2	2.8±0.9

* P<0.05 in comparison with physiological saline group treated i.c.v.

The effect of multiple applications of 6-OHDA on histological lesions in the central nervous system of animals with EAE - Judging from the number of perivascular mononuclear infiltrates in the tissue of the central nervous system, as expressed by the mean score, a statistically significant decrease was found in animals pretreated with 6-OHDA both i.c.v. and at the same time i.p. and i.c.v. (Table 3).

Table 3. The effect of multiple application of 6-OHDA on histological lesions in the central nervous system during experimental allergic encephalomyelitis (EAE) in rats. There were 9 animals in each group.

Group	Treatment	Way of application	Number of animals with histological lesions					Mean score (M±SD)	Percentage of rats with lesions
			0	+	+	+++	++++		
Experimental									
	6-OHDA	i.p.	0	0	3	1	5	3.2±0.9	100
	6-OHDA	i.c.v.	0	1	2	1	5	3.1±1.1*	100
	6-OHDA	i.c.v.+i.p.	0	2	1	1	5	3.0±1.3*	100
Controls									
	Physiological saline	i.p.	0	0	1	0	8	3.7±0.6	100
	—	i.c.v.	0	0	0	1	7	3.8±0.3	100

* $P<0.05$ in comparison with the physiological saline group treated i.c.v.

DISCUSSION

It was found in the present experiments that multiple pretreatment of the rats with 6-OHDA i.p. and/or i.c.v. produced a decrease in the severity of the general characteristics of EAE. This was evident from the slightly lower incidence of EAE, from the shorter duration of the disease and from the delay in onset of EAE. These changes were not statistically significant, except for the group of animals pretreated both i.p. and i.c.v. in which the duration of EAE was significantly decreased.

Similarly, the same type of pretreatment with 6-OHDA produced a statistically significant decrease in the intensity of clinical signs of EAE. Histological examination of perivascular mononuclear infiltrates in the tissues of the central nervous system showed a statistically significant decrease in animals pretreated with 6-OHDA i.c.v. and in a separate group both i.p. and i.c.v.

Thus, judging from all the criteria used in the present experiments, depletion of catecholamines by pretreatment with 6-OHDA produces a decrease in the severity of clinical signs and histological lesions in EAE.

Previous work has shown that a prerequisite for the development of EAE is the integrity of the central noradrenergic and dopaminergic pathways (Ovadia et al., 1989). A lesion in the hypothalamus has also been known to suppress development of EAE (Abramsky et al., 1987) and to decrease the titre of specific antibodies against myelin-basic-protein (MBP) (Wertman et al., 1985). Similarly, intracisternal injection of 6-OHDA has been shown to reduce the severity of EAE (Karpus et al., 1988). Our results are in agreement with all the aforementioned findings, but at variance with the results of Leonard et al., (1991) who has shown that pretreatment of newborn rats with 6-OHDA even potentiates the severity of EAE. Evidently, further experiments are needed in order to clarify the reason for this difference.

Our results indicate that chemical sympathectomy, as produced in the present experiments, may affect the development, severity and duration of experimental allergic encephalomyelitis. This shows that catecholamines might have a role in the development and clinical signs of EAE.

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DEJSTVO 6-HIDROKSIDOPAMINA NA EKSPERIMENTALNI ALERGIJSKI ENCEFALOMIJELITIS U PACOVA

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SADRŽAJ

U ovom radu je izučavana moguća uloga kateholamina na razvoj, tok i intenzitet eksperimentalnog autoimunog alergijskog encefalomijelitisa. U tom cilju je prethodno izazvano osiromašenje depoa kateholamina pomoću 6-hidroksidopamina (6-OHDA), jedne supstancije za koju se zna da prouzrokuje

razaranje adrenergičkih struktura. 6-OHDA je aplikovan pet puta intraperitonealno (i.p.) i/ili intracerebroventrikularno (i.c.v.) u periodu pre imunizacije. Neposredno posle toga pacovi su imunizovani emulzijom kičmene moždine zamorca. Dobijeni rezultati ukazuju da prethodno tretiranje 6-hidroksidopaminom, tj. osiromašenje kateholaminskih depoa, prouzrokuje supresivni efekt na razvoj, intenzitet i trajanje autoimunog encefalomijelitisa u pacova.