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THE EFFECT OF 6-HYDROXYDOPAMINE ON THE ARTHUS PHENOMENON AND DELAYED SKIN HYPERSENSITIVITY REACTIONS TO BOVINE SERUM ALBUMIN

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In order to produce chemical sympathectomy, Wistar rats were pretreated with 6-hydroxydopamine (6-OHDA), a substance known to produce general depletion of catecholamines from their stores. 6-OHDA was injected either intracerebroventricularly (i.c.v.) or intraperitoneally (i.p) into animals immunized with bovine serum albumin. The results obtained indicate that pretreatment with 6-hydroxydopamine, both after single and repeated injections, decreased the Arthus phenomenon and delayed skin hypersensitivity reaction.

Key words: 6-hydroxydopamine, adrenergic neurotransmitters, immune response, hypersensitivity, sympathectomy

INTRODUCTION

It has already been shown that many biologically active substances from the central nervous system participate in the regulation of the immune response (Janković et al., 1987, 1988; Horvat et al., 1991). On the other hand, some products of the immune system affect various physiological and homeostatic mechanisms in the central nervous system (Krueger et al., 1984.). Lymphocytes have been found to synthesize various biologically active substances, including hormones and opioids (Blalock and Smith, 1980; Blalock et al., 1985.).

Catecholamines are transmitters both in the central and autonomic nervous system. Previous results indicate that dopamine might be implicated in the immune response (Horvat et al., 1991.). It was therefore of interest to study the effects of chemical sympathectomy with 6-OHDA on skin hypersensitivity reactions in the rat.

MATERIALS AND METHODS

The experiments were performed on 8-week-old Wistar rats with a body mass of 200-250 g. A group of animals was implanted with a polyethylene cannula into the lateral brain chambers.

6-OHDA was injected either i.p (20 mg/kg b.m.) or i.c.v. (0,6 mg/kg b.m.in a volume of 10 μl). In a separate group of animals the drug was injected both i.p and i.c.v. A control group of animals was injected with saline in the same way and volume.

Pretreatment with 6-OHDA was done either as a single injection of the substance two days before immunization, or every second day (a total of 5 injections), the last injection being on the day of immunization.

The antigen used was crystalline bovine serum albumin (BSA), emulsified in Freund's complete adjuvant injected intradermally in a volume of 0,1 ml (0,5 mg of BSA) into the left hind paw.

Skin tests were performed 14 and 21 days after immunization. For that purpose BSA was injected intradermally in a dose 30 μg in 0,1 ml saline. The Arthus skin reaction was evaluated 4 hours later by measuring the diameter and oedema of reaction. Delayed skin hypersensitivity was evaluated after 24 hours by measuring the diameter of the reaction and the degree of induration.

RESULTS

The effect of a single injection of 6-OHDA on the Arthus phenomenon and delayed skin hypersensitivity. – The Arthus phenomenon was significantly depressed by pretreatment of the animal with 6-OHDA. This was observed when 6-OHDA was injected i.c.v. or i.p. This type of response was observed both 14 and 21 days after immunization. The results are presented in Table 1.

Table 1. Effect of a single injection of 6-OHDA on the Arthus phenomenon

Groups	Treatment	Route of administration	n	DAY 14			DAY 21		
				Positive reactions (%)	Mean diameter (mm±S.D.	Mean score (±S.D.)	Positive reactions (%)	Mean diameter (mm±S.D.)	Mean score (±S.D.)
Experimental	6-OHDA	i.p.	8	75	11.5±2.7	1.62±1.18	100	14.2±3.3	2.62±0.91[e]
	6-OHDA	i.c.v.	8	100	9.1±3.6[a]	1.50±0.53	100	9.0±2.5[c]	2.25±1.16
	6-OHDA	i.c.v.+i.p.	9	88	11.0±2.0[b]	1.66±0.86	100	11.8±3.6[d]	2.44±1.01
Control	Saline	i.p.	9	100	13.4±3.4	2.11±0.78	66	15.4±2.5	1.33±1.11
	Saline	i.c.v.	12	66	14.1±1.7	1.46±1.08	66	14.3±3.9	1.91±1.56

[a]p<0.001 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group
[b]p<0.001 -group treated with 6-OHDA i.c.v. +i.p. in comparison to i.c.v. saline treated group
[c]p<0.001 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group
[d]p<0.05 -group treated with 6-OHDA i.c.v. +i.p. in comparison to i.c.v. saline treated group
[e]p<0.01 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group

Delayed hypersensitivity reactions were also suppressed by pretreatment of the animal with a single injection of 6-OHDA either i.p or i.c.v. The immunosuppressive effect of 6-OHDA was evident by a decrease of oedema and induration, and also by diminished diameters of hypersensitivity reactions. The immunosuppressive effect of 6-OHDA was observed both 14 and 21 days after immunization. The results are shown in Table 2.

Table 2. Effect of a single injection of 6-OHDA on the delayed hypersensitivity reaction

Groups	Treatment	Route of administration	n	DAY 14			DAY 21		
				Positive reactions (%)	Mean diameter (mm±S.D.	Mean score (±S.D.)	Positive reactions (%)	Mean diameter (mm±S.D.)	Mean score (±S.D.)
Experimental	6-OHDA	i.p.	8	75	9.0±2.9[a]	1.00±0.75[c]	75	9.6±1.8	1.00+0.75[f]
	6-OHDA	i.c.v.	8	75	9.8±1.6[b]	1.37±1.06	75	11.0±1.1[d]	1.62±1.06
	6-OHDA	i.c.v.+i.p.	9	88	13.4±2.1	1.88±0.92	88	12.8±1.8[e]	1.56±1.01
Control	Saline	i.p.	9	88	13.2±2.2	1.88±0.92	88	9.6±2.1	1.88±0.92
	Saline	i.c.v.	12	58	12.7±3.3	1.33±1.23	66	15.3±3.4	1.75±1.42

[a]p<0.001 -group treated with 6-OHDA i.p. in comparison to i.p. saline treated group
[b]p<0.01 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group
[c]p<0.01 -group treated with 6-OHDA i.p. in comparison to i.p. saline treated group
[d]p<0.001 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group
[e]p<0.01 -group treated with 6-OHDA i.c.v. +i.p. in comparison to i.c.v. saline treated group
[f]p<0.01 -group treated with 6-OHDA i.p. in comparison to i.p. saline treated group

The effect of multiple injections of 6-OHDA on the Arthus phenomenon and delayed skin hypersensitivity. – As shown in Table 3, multiple injections of 6-OHDA (i.p., i.c.v.or both) produced a qualitative and quantitative immunosuppressive effect, similar to the response observed in animals given a single injection of the drug (Table 1 and 2). The Arthus phenomenon was significantly decreased both 14 and 21 days after immunization. A detailed analysis is presented in Table 3.

Table 3. Effect of multiple injections of 6-OHDA on the Arthus phenomenon

Groups	Treatment	Route of administration	n	DAY 14			DAY 21		
				Positive reactions (%)	Mean diameter (mm±S.D.	Mean score (±S.D.)	Positive reactions (%)	Mean diameter (mm±S.D.)	Mean score (±S.D.)
Experimental	6-OHDA	i.p.	10	80	11.1±3.2[a]	1.80±1.31	100	13.4±2.9[e]	2.70+1.05
	6-OHDA	i.c.v.	9	100	10.0±2.2[b]	1.44±0.52[c]	89	10.4±4.5[f]	1.33±0.70[h]
	6-OHDA	i.c.v.+i.p.	9	89	11.2±3.3	1.55±0.88[d]	78	14.4±3.6[g]	2.33±1.50
Control	Saline	i.p.	10	100	13.0±1.8	2.10±0.31	100	16.0±4.4	3.00±1.15
	Saline	i.c.v.	10	100	12.4±2.8	2.30±0.94	90	17.3±4.7	2.50±1.26

[a]p<0.05 -group treated with 6-OHDA i.p. in comparison to i.p. saline treated group
[b]p<0.01 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group
[c]p<0.01 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group
[d]p<0.05 -group treated with 6-OHDA i.c.v. +i.p. in comparison to i.c.v. saline treated group
[e]p<0.05 -group treated with 6-OHDA i.p. in comparison to i.p. saline treated group
[f]p<0.001 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group
[g]p<0.05 -group treated with 6-OHDA i.c.v. +i.p. in comparison to i.c.v. saline treated group
[h]p<0.01 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group

Delayed hypersensitivity reactions after multiple injections of 6-OHDA were also significantly attenuated, both 14 and 21 days after immunization (Table 4.).

Table 4. Effect of multiple injections of 6-OHDA on the delayed hypersensitivity reaction

Groups	Treatment	Route of administration	n	DAY 14			DAY 21		
				Positive reactions (%)	Mean diameter (mm±S.D.	Mean score (±S.D.)	Positive reactions (%)	Mean diameter (mm±S.D.)	Mean score (±S.D.)
Experimental	6-OHDA	i.p.	10	80	12.1±0.8[a]	2.10±1.19	60	11.4±2.3	1.20+1.22
	6-OHDA	i.c.v.	9	67	12.5±2.8[b]	0.77±0.66[c]	44	10.2±2.6[d]	0.66±0.86[f]
	6-OHDA	i.c.v.+i.p.	9	67	13.8±2.2	1.88±1.45	67	11.8±1.7[e]	1.33±1.22
Control	Saline	i.p.	10	80	15.3±3.6	2.10±1.44	100	11.7±2.4	1.50±0.70
	Saline	i.c.v.	10	100	14.7±3.3	2.40±0.84	100	13.5±2.0	1.50±1.08

[a]p<0.05 -group treated with 6-OHDA i.p. in comparison to i.p. saline treated group
[b]p<0.01 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group
[c]p<0.01 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group
[d]p<0.05 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group
[e]p<0.05 -group treated with 6-OHDA i.c.v. +i.p. in comparison to i.c.v. saline treated group
[f]p<0.001 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group

DISCUSSION

In the present experiments it was found that pretreatment of rats with 6-OHDA produced a significant depression in the immune response of the animals, as measured by the Arthus phenomenon and delayed skin hypersensitivity to BSA. This was observed both after single and multiple injections of 6-OHDA, and both 14 and 21 days after immunization with BSA.

Immunosuppression after 6-OHDA was more pronounced if the drug was injected i.c.v. or both i.c.v. and i.p. This holds true for the Arthus phenomenon, particularly 21 days after immunization.

Electrolytic lesions of the hypothalamus have been found to produce a depression of the delayed skin hypersensitivity, as well as a decrease in the humoral immune response (Janković i Isaković, 1973.). In mice, application of 6-OHDA was found to depress the skin contact senzitization with trinitrochlorobenzene (Madden et. al., 1989.). These authors indicated that 6-OHDA acts rather by producing a sympathetic denervation than by blocking the beta-adrenoceptors. As early as in 1964, Draškoci and Janković, demonstrated that catecholamines might be implicated in the immune response. Our experiments are in agreement with the previous results and suggestions and indicate that immunosuppression produced by 6-OHDA might be due to the missing noradrenaline link, caused by 6-OHDA pretreatment.

It is interesting that dopamine itself, as well as its precursor L-dopa, significantly depressed the Arthus phenomenon and delayed skin hypersensitivity (Horvat et al., 1991.). This raises the question of the mechanism of the imunosuppressive action of 6-OHDA. This substance has been observed to stop biosynthesis of catecholamines (Tranzer and Thoenen, 1967), eventually leading to "chemical sympathectomy". It seems reasonable to assume that an intact sympathetic system is necessary for immune responsiveness, whereas depletion of catecholamines depresses the immune response, as indeed found in our experiments. Nevertheless, the presented data do not exclude the possibility

of nonspecific toxic action of 6-OHDA, which may be quickly metabolized in the organism into toxic quinones. Further experiments are necessary in order to elucidate the mechanism of the coupling action of catecholamines on the immune inflammatory skin reactions to bovine serum albumin,

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DEJSTVO 6-HIDROKSIDOPAMINA NA ARTUSOV FENOMEN I NA REAKCIJU KASNE PREOSETLJIVOSTI PROTIV GOVEĐEG SERUM ALBUMINA

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SADRŽAJ

U cilju izazivanja hemijske simpatektomije, Wistar pacovi su prethodno tretirani 6-hidroksidopaminom, supstancijom za koju je poznato da prouzrokuje opšte osiromašenje depoa kateholamina iz njihovih depoa na taj način što zaustavlja njihovu sintezu. 6-Hidroksidopamin je ubrizgavan intracerebroventrikularno ili intraperitonealno. Ovaj agens je primenjen u obliku jednokratne injekcije ili u obliku višekratnog ubrizgavanja u toku 5 dana. Životinje su imunizovane bovinim serum albuminom.

Kožni testovi sa govedim serum albuminom izvođeni su dve i tri nedelje posle imunizacije. Dobijeni rezultati ukazuju da prethodno tretiranje sa 6-hidroksidopaminom, kako posle jednokratnog tako i višekratnog davanja, smanjuje Arthusov fenomen i reakcije kasne kožne preosetljivosti.