

## THE EFFECT OF 6-HYDROXYDOPAMINE ON PLAQUE-FORMING CELL RESPONSE AND HEMAGGLUTININ PRODUCTION IN THE RAT

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*Chemical sympathectomy was produced in Wistar rats by pretreatment with 6-hydroxydopamine (6-OHDA), a substance known to produce a general depletion of catecholamine stores. 6-OHDA was applied intracerebroventricularly (i.c.v.) or intraperitoneally (i.p.), either as a single injection or as 5 injections at two-day intervals. The animals were immunized with sheep erythrocytes. Both single and multiple pretreatment with 6-OHDA produced a significant decrease of plaque-forming cell response and hemagglutinin production to sheep erythrocytes. The results suggest a role for catecholamines in humoral immune reactions.*

*Key words: 6-Hydroxydopamine, catecholamines, immune response, antibody.*

### INTRODUCTION

Catecholamines have already been found to play a significant role in the immune response (Dražkoci and Janković, 1964; Besedovsky et al., 1979; Cross et al., 1986; Livnat et al., 1988). This includes effects on lymphocytes (maturation and proliferation), as well as activation and expression of receptors for various hormones and neurotransmitters. In a previous work we have shown that both single and repeated treatments with 6-OHDA, either i.c.v. or i.p. decrease the Arthus phenomenon and delay skin hypersensitivity to bovine serum albumin (Čupić et al., 1993). It was, therefore, of interest to study the effects of 6-OHDA on the number of plaque-forming cells (PFC) and amount of antibody circulating after immunization against sheep erythrocytes.

### MATERIALS AND METHODS

The experiments were performed in 8-week-old Wistar rats (body mass from 200 to 250 g). One group of animals, which had been implanted with polyethylene cannulae into the lateral brain ventricles, was injected with 6-OHDA (0.6 mg/kg b.m.). Another group of rats received 20 mg 6-OHDA/kg

b.m. i.p. A number of animals was given 6-OHDA simultaneously i.c.v. and i.p. The corresponding control groups were treated with saline.

6-OHDA was dissolved in 0.1 per cent ascorbic acid in saline, and injected i.c.v. or i.p. only once, or 5 times every second day. Two days after a single injection or on the last day of a series of injections, the animals were immunized. For this purpose,  $5 \times 10^9$  sheep erythrocytes were injected intraperitoneally, and four days later the PFC response was determined, using a modification of the method described by Cunningham and Szenberg (1968). Hemagglutinin titers in sera of immunized animals were determined by means of a microhemagglutination reaction.

## RESULTS

The effect of a single injection of 6-OHDA on the number of plaque-forming cells and on antibody production against sheep erythrocytes. Pretreatment of the animal with 6-OHDA i. p. and/or i.c.v. produced a marked decrease in the number of plaque-forming cells present after immunization. No matter which route of administration of 6-OHDA was employed, the number of PFC was statistically significantly lower than in the corresponding saline-treated controls (Table 1).

Table 1. Effect of a single injection of 6-ohda on plaque-forming cell (pfc) response and antibody production against sheep red blood cells.

Groups	Treatment	Route of administration	n	PFC/10 <sup>6</sup> cells ( $\bar{X} \pm SD$ )	Antibody titer ( $\log_2$ ): ( $\bar{X} \pm SD$ )
Experimental	6-OHDA	i.p.	10	353.0 $\pm$ 253.0 <sup>a</sup>	9.2 $\pm$ 1.4
	6-OHDA	i.c.v.	10	372.0 $\pm$ 285.7 <sup>b</sup>	8.4 $\pm$ 0.3 <sup>d</sup>
	6-OHDA	i.c.v.+i.p.	10	283.0 $\pm$ 229.9 <sup>c</sup>	10.5 $\pm$ 1.7
Control	Saline	i.p.	11	635.0 $\pm$ 344.0	9.5 $\pm$ 1.6
	Saline	i.c.v.	10	618.0 $\pm$ 344.0	9.1 $\pm$ 1.4

<sup>a</sup> $p < 0.01$  -group treated with 6-OHDA i.p. in comparison to i.p. saline treated group

<sup>b</sup> $p < 0.05$  -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group

<sup>c</sup> $p < 0.01$  -group treated with 6-OHDA i.c.v. +i.p. in comparison to i.c.v. saline treated group

<sup>d</sup> $p < 0.05$  -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group

Antibody titers observed in animals given a single injection of 6-OHDA were also lower in comparison with those found in saline-treated controls (Table 1) but the effect was not statistically significant after the i.c.v. route of administration.

The effect of multiple injections of 6-OHDA on the number of plaque-forming cells and on antibody production against sheep erythrocytes. Pretreatment of animals with multiple i.c.v. or i.p. injections of 6-OHDA produced a decrease in the number of plaque-forming cells present after immunization (Table 2).

Multiple pretreatment with 6-OHDA also produced a pronounced suppression of hemagglutinin production in animals treated i.p. or i.c.v. (Table 2).

Table 2. Effect of multiple injections of 6-ohda on plaque-forming cell (pfc) response and antibody production against sheep red blood cells.

Groups	Treatment	Route of administration	n	PFC/10 <sup>6</sup> cells ( $\bar{X} \pm$ SD)	Antibody titer (log <sub>2</sub> ): ( $\bar{X} \pm$ SD)
Experimental	6-OHDA	i.p.	10	480.0 $\pm$ 354.0 <sup>a</sup>	7.7 $\pm$ 1.6 <sup>c</sup>
	6-OHDA	i.c.v.	9	640.0 $\pm$ 382.9	7.0 $\pm$ 1.7 <sup>d</sup>
	6-OHDA	i.c.v.+i.p.	7	406.0 $\pm$ 362.8 <sup>b</sup>	6.2 $\pm$ 3.1 <sup>e</sup>
Control	Saline	i.p.	10	959.0 $\pm$ 691.4	8.9 $\pm$ 1.3
	Saline	i.c.v.	8	1102.5 $\pm$ 854.9	8.1 $\pm$ 1.1

<sup>a</sup>p<0.01 -group treated with 6-OHDA i.p. in comparison to i.p. saline treated group

<sup>b</sup>p<0.01 -group treated with 6-OHDA i.c.v.+i.p. in comparison to i.c.v. saline treated group

<sup>c</sup>p<0.01 -group treated with 6-OHDA i.p. in comparison to i.p. saline treated group

<sup>d</sup>p<0.05 -group treated with 6-OHDA i.c.v. in comparison to i.c.v. saline treated group

<sup>e</sup>p<0.01 -group treated with 6-OHDA i.c.v.+i.p. in comparison to i.c.v. saline treated group

## DISCUSSION

The present experiments revealed that both single and multiple pretreatment with 6-OHDA produced a significant depression in the response of plaque-forming cells and the production of antibody against sheep erythrocytes. The immunosuppression occurred when 6-OHDA was injected intraperitoneally and/or intracerebroventricularly.

6-OHDA has been already found to produce a decrease of PFC response in the developing chick embryo (Janković et al., 1990). This type of response was dependent on the dose of 6-OHDA used. The immunosuppressive effect of 6-OHDA has also been observed in adult mice treated with 6-OHDA on the day of immunization or two days later (Kasahara et al., 1977). Interestingly enough, there was no immunosuppression if 6-OHDA was applied 2–7 days before immunization. These results suggest that the time relationship between the pretreatment with 6-OHDA and immunization of the animals might be critical for the immunomodulatory activity of 6-OHDA.

The primary immune response and the development of an immunological memory have been shown to depend on the catecholamine content in various structures of the central nervous system (Cross et al., 1986). It has been found that the noradrenaline level in the hypothalamus, striatum, midbrain and pons is significantly decreased 48 hours after 6-OHDA pretreatment (Kostrzewa and Jacobowitz, 1974). Depending on the degree of noradrenaline depletion in the central nervous system, the animals were divided into "high" and "low" immunological responders (Besedovsky et al., 1983).

As already indicated, multiple treatment with 6-OHDA produced a significant decrease in the antibody titer in rats immunized with sheep erythrocytes. The effect after a single injection of 6-OHDA, was not statistically significant

indicating that a higher degree of catecholamine depletion is necessary for depression of antibody production.

It can be concluded, therefore, that intact catecholamine stores in the central and autonomic nervous system are prerequisites for a normal immunological response of the rat to sheep erythrocytes. Catecholamine depletion by pretreatment with 6-OHDA renders the animals incapable of responding adequately to antigen, as indicated by a decrease in the number of plaque-forming cells and antibody titers, as observed in the present study.

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#### UTICAJ 6-HIDROKSIDOPAMINA NA STVARANJE HEMOLITIČKIH PLAKA I PRODUKCIJU HEMAGLUTININA U PACOVA

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#### SADRŽAJ

Hemijska simpatetomija je izazivana prethodnim tretiranjem životinje 6-hidroksidopaminom (6-OHDA), supstancijom za koju se zna da prouzrokuje opšte osiromašenje depoa kateholamina. Ova supstancija je primenjivana intracerebroventrikularno (0.6 mg/kg) ili intraperitonealno (20 mg/kg), bilo kao

pojedinačna injekcija, bilo da su injekcije primenjivane svaki drugi dan (ukupno 5 injekcija). Životinje su imunizovane ovčijim eritrocitima. Pojedinačan i multipli pretretman pacova sa 6-OHDA prouzrokuje značajno smanjenje PFC odgovora. Ista procedura davanja 6-OHDA dovodi do značajnog smanjenja produkcije antitela protiv ovčijih eritrocita. Rezultati ukazuju na moguću ulogu kateholamina u humoralnim imunim reakcijama.