

# The Effect of Dopaminergic Agents on Cell-Mediated Immune Response in Mice

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## Summary

The aim of the study was to determine the effect of selective dopaminergic agents [(±)-SKF-81297 (D<sub>1</sub> agonist), R(-)-2,10,11-trihydroxy-N-propyl-noraporphine (D<sub>2</sub> agonist), pergolid (D agonist), R(+)-SCH-23390 (D<sub>1</sub> antagonist), S(-)-eticlopride (D<sub>2</sub> antagonist) and cis-(Z)-flupenthixol (D antagonist)] on cell-mediated immune response *in vivo* and *in vitro* and to verify the presence of dopamine receptors on murine splenocytes. The tested dopaminergic compounds exhibited a pronounced inhibitory effect on T-dependent immunity. They suppressed alloantigen-induced immune response *in vivo* and *in vitro*, IL-2 production was also markedly reduced. No substantial difference was found between the effect of dopamine agonists and antagonists or among ligands of subtypes of dopamine receptors. The effect of dopaminergic agents *in vitro* indicates a direct interaction with immunocompetent cells at the peripheral level. As the binding studies did not confirm the presence of dopamine receptors on splenocytes, the immunosuppressive efficacy of dopaminergic agents does not seem to be mediated *via* specific dopamine receptors.

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## Key words

Dopaminergic agents – Cellular immune response – Dopamine receptors – Immunomodulation

## Introduction

The neuroendocrine and immune systems are essential for the maintenance of homeostasis and integrity of organisms. Their functions are connected and bidirectionally regulated (Plata-Salamán 1991, Blalock 1994). The immune system is capable of influencing the activity of central nervous and endocrine systems through the release of cytokines, hormones and neuropeptides (Blalock 1992, Nistico *et al.* 1993, Mandrup-Poulsen *et al.* 1995). On the other hand, the immune response is modulated at the humoral and neuroanatomical level by products of the neuroendocrine system such as neuropeptides and neurotransmitters (Weigent and Blalock 1989, Berczi 1989). The primary and secondary lymphoid organs are innervated by the autonomic nervous system (Felten *et*

*al.* 1992). The presence of receptors for hormones, neuropeptides and neurotransmitters on the surface of immunocompetent cells indicates that these bioactive substances exert immunomodulatory activity (Cavagnaro and Lewis 1989, Homo-Delarche and Dardenne 1993).

Clinical and experimental observations have proved that the dopaminergic system modulates the activity of immunocompetent cells. The disturbances of central dopaminergic transmission in schizophrenia (Rabin *et al.* 1989) and Parkinson's disease (Fisher *et al.* 1991), as well as nigrostriatal and mesolimbic dopaminergic lesions (Deleplanque *et al.* 1994), resulted in dysregulation of immune functions. Similarly, dopamine has been referred to possess immunomodulatory properties (Boukhris *et al.* 1987, Masuda *et al.* 1995).

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Results were preliminary presented at "CNS – Advance in Research of Normal and Neoplastic Cells" which was held in Brno (April 25, 1996) as the satellite minisymposium of the 42nd International Congress of the European Tissue Culture Society (Panajotova 1996).

The aim of the study was to determine the effect of the dopaminergic system on the cellular immune response *in vivo* and *in vitro* using selective dopamine agonists and antagonists and to verify the presence of dopamine receptors on splenocytes.

## Methods

### Animals

Inbred C57BL10/ScSn female mice (for the graft-versus-host reaction, IL-2 production and binding studies), F1 female hybrids C57BL10/ScSn x A/PHA (for graft-versus-host reaction), inbred C3H and DBA1 female mice (for mixed lymphocyte reaction) were used. The mice weighed 20–25 g and were purchased from Velaz, Prague or from the Czech Academy of Sciences (DBA1 mice).

### Drugs

(±)-6-chloro-PB hydrobromide [(±)-SKF-81297, D<sub>1</sub> agonist], R(-)-2,10,11-trihydroxy-N-propyl-noraporphine hydrobromide [D<sub>2</sub> agonist], pergolid methane-sulfonate [D agonist], R(+)-SCH-23390 hydrochloride [D<sub>1</sub> antagonist], S(-)-eticlopride hydrochloride [D<sub>2</sub> antagonist] and cis-(Z)-flupenthixol dihydrochloride [D antagonist] were obtained from RBI, USA. The drugs were administered orally in doses of 0.1 and 1.0 mg/kg in solution. *In vitro*, the drugs were tested in concentrations of 1 µg/ml, 1 ng/ml and 1 pg/ml.

### Graft-versus-host reaction (GVHR)

Local graft-versus-host reaction was elicited by transplantation of C57BL10 murine splenocytes into left hind footpad of F1 hybrids C57BL10 x A/PHA on day 0 (Twist and Barne 1973). The animals were sacrificed after 7 days, popliteal lymph nodes were removed and weighed. GVHR was expressed as the difference between weight of left and right popliteal lymph nodes (GVHR index). The test drugs were administered on days 0–4.

### Bidirectional mixed lymphocyte reaction (MLR)

The test was performed *in vitro* (Bradley 1980). C3H splenocyte suspensions were cultivated in microplates with test drugs and with DBA1 splenocytes (ratio 8:1) at 37 °C and 3.5 % CO<sub>2</sub>. After 96 h the cells were labelled with [<sup>3</sup>H]thymidine, incubated for 6 h and harvested. The amount of incorporated precursor was measured on a liquid scintillation analyzer and expressed as dpm.

### Interleukin 2 production

The spleens from C57BL10 mice were suspended in a tissue culture medium RPMI-1640. Primary tissue cultures (concentration 2 x 10<sup>6</sup> cells/ml) were incubated with concanavalin A (Con A, final

concentration 6 µg/ml) and with test substances at 37 °C and 3.5 % CO<sub>2</sub>. The supernatants were collected after 48 h. The IL-2 concentrations in supernatants were determined spectrophotometrically using commercial kit Intertest-2X (Genzyme, USA). The concentration of the test cytokine was calculated from a standard curve and expressed in pg/ml.

### Binding study

The suspension of splenocytes from 50 C57BL10 mice was supplemented with buffer A (20 mM HEPES-HCl, pH=7.4) to 20 ml and centrifuged 10 min at 500 x g. The supernatant was removed and the procedure was repeated. The sediment was resuspended in 2 ml of buffer B (50 mM Tris-HCl, 4 mM CaCl<sub>2</sub>, 0.1 % ascorbic acid and 10 mM pargyline). The splenocytes were homogenized, the homogenate was supplemented to 20 ml and centrifuged at 40 000 x g. The supernatant was removed and the pellet was stored at -80 °C.

The binding studies were carried out at 25 °C in 1 ml of buffer B. The samples were incubated with 2.5 mM [<sup>3</sup>H]SCH-23390 (D<sub>1</sub> antagonist, specific activity 182 dpm/fmol, Amersham, U.K.) or with 2.5 mM [<sup>3</sup>H]spiperone (D<sub>2</sub> antagonist, specific activity 253 dpm/fmol, Amersham, U.K.) for 60 min in the dark. The incubation was stopped with 3 ml of ice-cold water. The content of vials was filtered and the radioactivity was measured on a liquid scintillation analyzer (TRI-CARB 2200 CA, Packard Canberra, USA).

## Results

*In vivo*, all dopaminergic agents administered repeatedly in doses of 0.1 and 1 mg/kg p. o. tended to suppress the local graft-versus-host reaction. The most pronounced dose-dependent inhibition was obtained after administration of both doses of D antagonist flupenthixol and the higher dose of SCH-23390 (D<sub>1</sub> antagonist). The results are shown in Table 1.

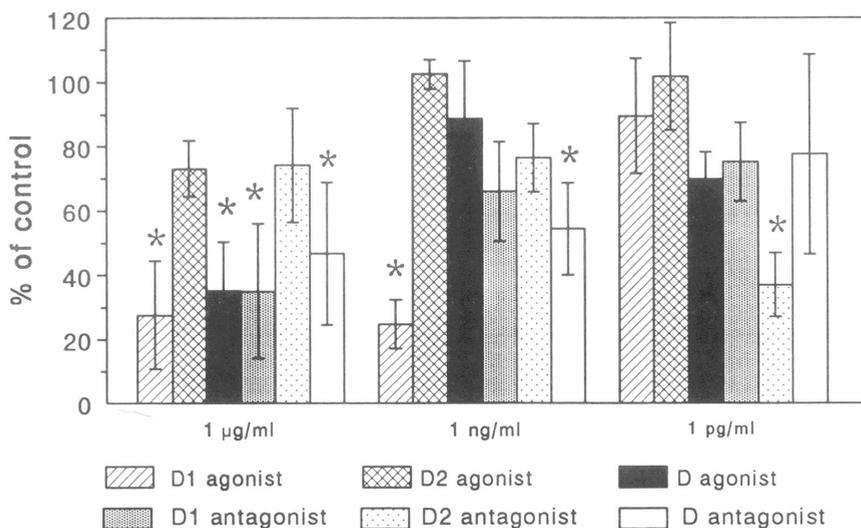
The test substances also exhibited immunosuppressive activity *in vitro* (Figs 1 and 2). Most of the dopaminergic agents significantly inhibited the bidirectional mixed lymphocyte reaction in a concentration of 1 µg/ml, the D<sub>2</sub> antagonist and agonist in this concentration decreased MLR non-significantly. All substances markedly reduced the IL-2 production of activated splenocytes.

The binding studies with [<sup>3</sup>H]SCH-23390 and [<sup>3</sup>H]spiperone did not reveal D<sub>1</sub> and D<sub>2</sub> receptors on murine splenocytes. Although both radioligands were used in high concentrations and the membrane concentration was increased, the difference between binding of radioligands with or without a non-labelled competitor was not found.

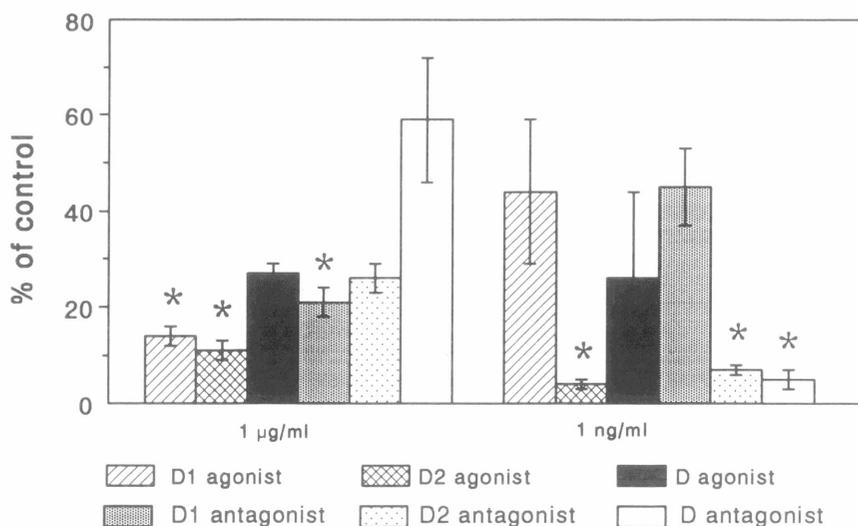
**Table 1.** The effect of dopaminergic agents on graft-versus-host reaction (GVHR). The test substances were orally administered repeatedly on days 0–4. Splenocyte transplantation was carried out on day 0 and the GVHR index was determined on day 7.

Group	Dose (mg/kg p.o.)	GVHR (mean $\pm$ S.E.M.)	% of inhibition
Control	–	1.39 $\pm$ 0.24	–
D agonist	0.1	0.84 $\pm$ 0.15	39.6
	1.0	1.12 $\pm$ 0.26	19.4
D antagonist	0.1	0.70 $\pm$ 0.12*	49.6
	1.0	0.45 $\pm$ 0.15*	67.6
Control	–	2.12 $\pm$ 0.56	–
D <sub>1</sub> agonist	0.1	1.16 $\pm$ 0.17	45.3
	1.0	1.36 $\pm$ 0.32	35.8
D <sub>2</sub> agonist	0.1	1.10 $\pm$ 0.35	48.1
	1.0	1.63 $\pm$ 0.43	23.1
Control	–	2.01 $\pm$ 0.49	–
D <sub>1</sub> antagonist	0.1	1.31 $\pm$ 0.23	34.8
	1.0	0.64 $\pm$ 0.17*	68.2
D <sub>2</sub> antagonist	0.1	1.36 $\pm$ 0.41	32.3
	1.0	1.80 $\pm$ 0.49	10.4

D agonist - pergolid; D antagonist - cis-(Z)-flupenthixol; D<sub>1</sub> agonist - ( $\pm$ )-6-chloro-PB; D<sub>2</sub> agonist - R(-)-2,10,11-trihydroxy-N-propyl-noraporphine; D<sub>1</sub> antagonist - R(+)-SCH-23390; D<sub>2</sub> antagonist - S(-)-eticlopride. Statistical analysis was performed using Student's t-test at 5 % level of significance. The results are expressed as mean  $\pm$  S.E.M. and as % of inhibition related to the control group. The significant differences ( $p < 0.05$ ) are indicated by asterisks. Each group contained 8–11 animals.



**Fig. 1.** The effect of dopaminergic agents on bidirectional mixed lymphocyte reaction (MLR). The murine C3H splenocytes were incubated with DBA1 splenocytes (ratio 8:1) and with the test substances. [<sup>3</sup>H]thymidine incorporation was evaluated after 96 h. The samples were tested in 6 aliquots and the results are expressed as % of control (i. e. splenocytes incubated without test substance)  $\pm$  S.E.M.. The statistical analysis was carried out using Student's t-test at 5 % level of significance and significant differences between control and experimental values are indicated by asterisks.



**Fig. 2.** The effect of dopaminergic agents on IL-2 production. The murine B10 splenocytes were incubated with Con A (final concentration 6 µg/ml) and the test substances. IL-2 concentrations were determined in supernatants after 48 h. The samples were tested in triplicates and the results are expressed as % of control (splenocytes incubated without test substance) ± S.E.M. The statistical analysis was carried out using Student's *t*-test at 5% level of significance and significant differences between control and experimental values are indicated by asterisks.

## Discussion

The selective dopaminergic agents exhibited a pronounced suppressive effect on the T-dependent immune response at systemic and peripheral levels. In the *in vivo* model of alloantigen-driven immune response (local GVHR), the drugs decreased the GVHR index to a varying extent. An inhibitory effect was also found in test of alloantigen-induced splenocyte proliferation *in vitro* (mixed lymphocyte reaction). Moreover, the incubation of Con A-activated splenocytes with the dopaminergic agents markedly reduced IL-2 production. The decrease of IL-2 production could represent one of the mechanisms inhibiting cell-mediated immune responses such as the graft-versus-host or mixed lymphocyte reactions (Puré *et al.* 1988, Antin and Ferrara 1992).

Literary data on the immunomodulatory effect of dopaminergic agents on the cell-mediated immune response are scarce. The repeated administration of dopamine antagonist haloperidol resulted in marked suppression of DTH in mice (Descotes 1986). However, in an other study on mice (Roudebush *et al.* 1991), selective dopamine antagonists haloperidol, sulpiride and metoclopramide did not affect systemic GVHR and DTH significantly. The dopamine agonist, bromocriptine, exhibited an immunosuppressive effect *in vivo* (Nagy *et al.* 1983). Recently, bromocriptine was also reported to inhibit the activity of human T lymphocytes *in vitro* – it reduced IL-2 production and MLR (Morikawa *et al.* 1994). Moreover, the immunosuppressive activity of bromocriptine appeared

to be independent of its hypoprolactinaemic effect. It thus seems that the resulting immunomodulatory effect of dopaminergic agents depends on the experimental conditions, especially on drug dosage and mode of administration.

In the present study, we used selective agonists and antagonists of D<sub>1</sub> and D<sub>2</sub> receptors (Sunahara *et al.* 1993) but, interestingly, we failed to find any substantial difference between their immunomodulatory activity. Their direct immunosuppressive effect *in vitro* indicates that they are able to influence the cellular immune response independently of the neuroendocrine system.

The binding studies did not reveal the presence of D<sub>1</sub> or D<sub>2</sub> receptors on murine splenocytes. Besides the CNS, dopamine receptors are also present in several peripheral organs such as the adrenal gland, kidneys and the heart (Civelli 1995, O'Connell 1996). The distribution of dopamine receptors on immunocompetent cells has not been studied in detail. Hitherto, dopamine receptors have only been proved convincingly on thymocytes (Ricci *et al.* 1995). The results document a pronounced CNS-independent immunosuppressive effect of selective dopaminergic agents on the cell-mediated immune response. Our study did not provide evidence, either pharmacologically or by binding studies, that dopamine receptors are present on the surface of immunocompetent cells in the spleen. Thus, the direct interaction of dopaminergic agents need not be mediated *via* dopamine receptors.

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