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Effects of nitric oxide synthase inhibition on diethylstilbestrol-induced hyperprolactinaemia and pituitary tumourigenesis in rats

Wpływ zahamowania syntazy tlenku azotu na wywołane dietylstilbestrolem hiperprolaktynemię i rozwój guza przysadki

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Abstract

Introduction: The overexpression of nitric oxide synthase (NOS) has been found in tumours, including pituitary adenomas. It has also been found that NOS is overexpressed in human spontaneous pituitary adenomas. The question arises whether NOS and its product, nitric oxide (NO), are involved in pituitary tumourigenesis. To investigate this question, in the present paper we examine the effects of NOS inhibition on the development of diethylstilbestrol (DES)-induced prolactin–secreting pituitary tumours in rats.

Material and methods: Thirty male Fisher 344 rats, four weeks old, were submitted to subcutaneous implantation of a silastic capsule containing DES (10 mg/capsule) or of an empty capsule. Six weeks after implantation, some of the DES-treated animals received a NOS inhibitor, N-nitro-l-arginine methyl ester (NAME), 1 mg/mL, in their drinking water, for the subsequent 14 days. Eight weeks after the implantation, all the animals were sacrificed, their pituitaries were weighed, and samples of heart blood were collected for prolactin (PRL) and vascular endothelial growth factor (VEGF) measurements.

Results: It was found that DES implantation significantly increased pituitary mass, as well as PRL and VEGF concentrations in blood serum. On the other hand, the administration of NAME did not affect significantly either VEGF concentration or pituitary mass. On the other hand, it did induce a further increase in PRL levels.

Conclusions: These findings indicate that NO is involved in oestrogen-induced hyperprolactinaemia, but does not play a crucial role in oestrogen-induced pituitary tumourigenesis. **(Pol J Endocrinol 2012; 63 (2): 115–118)**

Key words: *nitric oxide, prolactin, VEGF, oestrogen-induced pituitary tumour*

Streszczenie

Wstęp: W licznych nowotworach, w tym także gruczolakach przysadki, stwierdzono zwiększoną ekspresję syntazy tlenku azotu (NOS). Powstaje pytanie, czy NOS oraz jej produkt — tlenek azotu (NO) biorą udział w patogenezie guzów przysadki. W celu zbadania tego zagadnienia autorzy niniejszej pracy prześledzili wpływ zahamowania aktywności NOS na rozwój prolaktynowego guza przysadki wywołanego dietylstilbestrolem (DES) u szczurów.

Materiał i metody: Czterotygodniowym szczurom samcom szczepu Fisher 344 implantowano podskórnie silastikowe kapsułki zawierające 10 mg DES w kapsułce lub puste kapsułki. Sześć tygodni po implantacji część zwierząt, którym implantowano kapsułki z DES, otrzymywała inhibitor NOS — ester metylowy N-nitro-l-argininy (NAME) w wodzie do picia, w stężeniu 1 mg/ml, przez 14 dni. Osiem tygodni po implantacji kapsułek zwierzęta uśmiercono, pobrano i zważono przysadki oraz próbki krwi z serca w celu oznaczenia stężeń prolaktyny (PRL) i czynnika wzrostu śródbłonków naczyniowych (VEGF).

Wyniki: Stwierdzono, że implantacja DES znamiennie zwiększa masę przysadki oraz stężenia PRL i VEGF w surowicy krwi. Równoczesne podawanie NAME nie ma istotnego wpływu na masę przysadki ani stężenie VEGF, natomiast powoduje dalszy wzrost stężenia PRL.

Wnioski: Uzyskane wyniki wskazują, że NO wpływa na hiperprolaktynemię indukowaną DES, ale nie odgrywa istotnej roli w rozwoju guza prolaktynowego indukowanego estrogenem. **(Endokrynol Pol 2012; 63 (2): 115–118)**

Słowa kluczowe: *tlenek azotu, prolaktyna, VEGF, guz przysadki wywołany estrogenem*

Introduction

Nitric oxide (NO), a molecule generated from arginine by an enzyme called nitric oxide synthase (NOS), plays an important role as a messenger involved in numerous regulatory functions within the living organism. The best known role of NO is relaxation of the blood vessels [1]. More recently, overexpression of NOS has been found in several human tumours [2–6] including pituitary adenomas [7, 8]. In a murine glioma model, NOS inhibition results in slowing tumour growth [9]. Overexpression of NOS is associated with poor sur-

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vival in patients suffering from cervical cancer [10] and oestrogen receptor-negative breast cancer [11]. On the other hand, inducible NOS expression is considered to be a favourable prognostic marker in early-stage cervical cancer [12]. It has also been found that NOS is overexpressed in experimental diethylstrilbestrol (DES)-induced prolactinomas in rats [8, 13] compared to normal rat anterior pituitary gland.

The question arises as to whether NOS and its product, NO, are involved in pituitary tumourigenesis. To test this question, in the present paper we examined the effects of NOS inhibition on the development of DES-induced prolactin-secreting pituitary tumours in rats. Numerous studies have shown the increase of vascular endothelial growth factor (VEGF) expression in tumours and a correlation between VEGF levels and microvascular density [14, 15]. The development of pituitary adenomas, like other solid tumours, also depends on neo-angiogenesis. VEGF levels have been found to be elevated in patients with pituitary tumours [16–18] and the administration of anti-angiogenic agents have inhibited the growth of experimental oestrogen-induced rat prolactinoma [19]. On the other hand, vascularisation of pituitary adenomas is poorer than that of a normal pituitary, and VEGF levels do not correlate with microvascular density in human pituitary adenomas [17]. In pituitary adenomas, VEGF receptors have been found to be expressed not only in endothelial cells of intratumoural blood vessels, but also in tumoural cells [20]. It has been suggested that VEGF in pituitary adenomas acts not only as a stimulator of angiogenesis, but directly promotes growth of tumoural cells. The recent *in vitro* experiments of Zatelli et al. [21], who found that the somatostatin analogue pasireotide inhibits the viability of pituitary adenoma cells in culture acting via VEGF suppression, confirm this hypothesis. Because of that, we also examined the effect of NO inhibition on VEGF concentrations in blood serum during DES-induced pituitary tumourigenesis.

Material and methods

Induction of pituitary tumours in rats

Thirty male Fisher 344 rats, four weeks old, were submitted under ketamine anaesthesia to subcutaneous implantation of a silastic capsule containing DES (10 mg/capsule, 20 animals) or of an empty capsule (control = ten rats). Six weeks after implantation, ten DES-treated animals received a NOS inhibitor, N-nitro-l-arginine methyl ester (NAME), 1 mg/mL in their drinking water, for the subsequent 14 days. Another DES-treated group (ten rats) was given water that did not contain NAME. Eight weeks after the implantation, all the animals were sacrificed by decapitation, the pituitaries were weighed, and samples of heart blood were collected for prolactin and vascular endothelial growth factor (VEGF) measurements. The experiment was approved by the Ethical Committee for Animal Experimentation in Lodz (decision Ł/BD/150).

Prolactin assay

Prolactin (PRL) concentrations in the blood serum were measured using rat prolactin (rPRL) [125I] — Biotrak Assay System with Magnetic Separation (Amersham, UK, Code: RPA 553). The assay sensitivity was: 0.7 ng/mL and the coefficients of inter- and intra-assays variations were 8.3% and 10.5% respectively.

VEGF assay

Vascular endothelial growth factor (VEGF) was measured in the blood serum using ELISA mouse/rat VEGF assay kits (Quantikine, R&D Systems, Minneapolis, MN, USA).

Statistical analysis

Statistical analysis was performed by means of ANOVA test followed by LSD test.

Results

The results are presented in Figures 1–3. As can be seen there, DES implantation induced an approximately three-fold increase in pituitary mass (DES

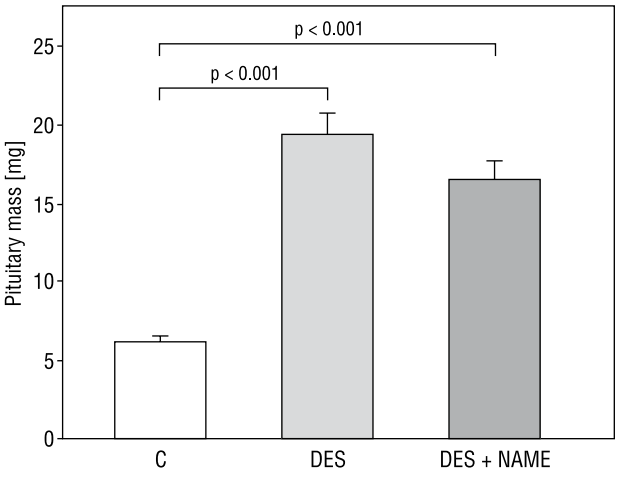

Figure 1. *Effects of diethylstilbestrol alone (DES), and DES + N-nitro-l-arginine methyl ester (NAME), on the anterior pituitary mass in male Fisher 344 rats (means ± SEM); C — control*

Rycina 1. *Wpływ samego dietylstilbestrolu (DES) oraz DES łącznie z estrem metylowym N-nitro-l-argininy (NAME) na masę przysadki szczurów samców szczepu Fisher 344 (średnie ± SEM); C — grupa kontrolna*

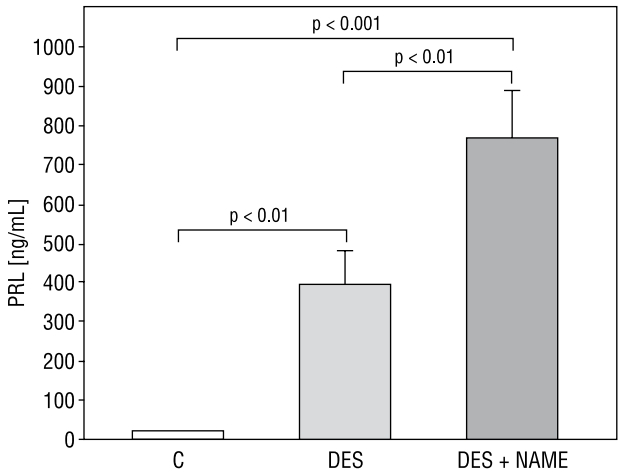

Figure 2. *Effects of diethylstilbestrol alone (DES), and DES + + N-nitro-l-arginine methyl ester (NAME), on prolactin (PRL) blood serum concentrations in male Fisher 344 rats (means ± SEM); C — control*

Rycina 2. *Wpływ samego dietylstilbestrolu (DES) oraz DES łącznie z estrem metylowym N-nitro-l-argininy (NAME) na stężenia prolaktyny (PRL) w surowicy krwi szczurów samców szczepu Fisher 344 (średnie ± SEM); C — grupa kontrolna*

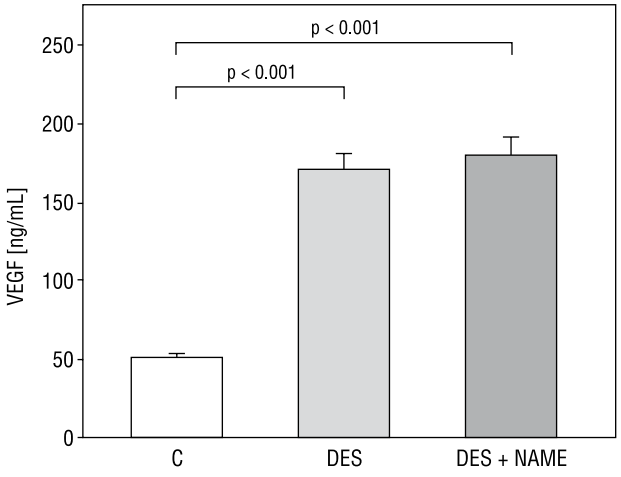

Figure 3. *Effects of diethylstilbestrol alone (DES), and DES + + N-nitro-l-arginine methyl ester (NAME), on vascular endothelial growth factor (VEGF) blood serum concentrations in male Fisher 344 rats (means ± SEM); C — control*

Rycina 3. *Wpływ samego dietylstilbestrolu (DES) oraz DES łącznie z estrem metylowym N-nitro-l-argininy (NAME) na stężenia czynnika wzrostu śródbłonka naczyniowego (VEGF) w surowicy krwi szczurów samców szczepu Fisher 344 (średnie ± SEM); C — grupa kontrolna*

18.99 ± 1.26 mg *vs.* control 6.15 ± 0.36 mg, $p < 0.001$, Figure 1) as well as a 27-fold increase in PRL concentration in blood serum (DES 356 ± 86.8 ng/mL *vs.* 13.1 ± 3.5 ng/mL, $p < 0.001$, Figure 2). Moreover, the mean blood serum concentration of VEGF was increased approximatively three-fold in DES-implanted animals (171.7 ± 10.4 *vs.* 51.8 ± 6.0 pg/mL, $p < 0.001$, Figure 3). The simultaneous administration of the NOS inhibitor, NAME, did not affect VEGF concentrations (DES 171.7 ± 10.4 *vs.* DES+NAME 180.8 ± 11.6 pg/mL) and slightly, but not significantly, decreased the pituitary mass (DES + NAME 16.5 ± 1.31 mg *vs.* DES 18.99 ± 1.26 mg, Figure 1), but induced a further increase in PRL level (DES + NAME 769 ± 120 ng/mL *vs.* DES 356 ± 86.8 ng/mL, $p < 0.01$, Figure 2).

Discussion

As expected, DES implantation resulted in sharp increases of pituitary mass and PRL secretion. We found in our previous studies that prolactin cells undergo hyperplasia and form the vast majority of the adenomatous structure [13]. A sharp increase concerns also the vascular endothelial growth factor (VEGF), which confirms its involvement in pituitary tumourigenesis via pro-angiogenic, and, possibly, direct growth-promoting action [18, 19, 21]. The observed overexpression of NOS in both spontaneous human pituitary adenomas and in oestrogen-induced experimental rat pituitary tumours, suggested a role of NO in tumoural pituitary tumourigenesis [8]. Such a possibility is supported by a study showing the antiapoptotic effect of NO on the anterior pituitary cells [22]. Although we did not measure directly the activity of NOS in our study, the same dosage of NAME has been found to decrease NOS activity in the rat thyroid gland by more than 75% [23]. However, the inhibition of NO generation affected neither pituitary growth nor serum VEGF concentrations, as was shown in the present study. Interestingly, NO inhibition did not suppress, and in fact even enhanced, DES-induced hyperprolactinaemia. Earlier studies concerning the relation between NO and PRL secretion resulted in divergent effects, depending on the experimental models. NO is found either to enhance [24, 25] or to inhibit [26–28] PRL secretion. The mechanism of the latter effect, which corroborates with our study, may involve the PRL-inhibiting action of dopamine. It has been shown that dopamine's effect on PRL release *in vitro* is suppressed by NO inhibitors [23]. Moreover, NO affects the central dopaminergic system; for instance, the NO synthesis inhibitor has been shown to enhance the amphetamine-induced dopamine release in the neostriatum [29].

Conclusions

Summing up, although NO synthesis inhibition enhances oestrogen-induced hyperprolactinaemia, NO seems not to play an important role in oestrogen-induced pituitary hyperplasia and, possibly, tumourigenesis.

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Conflict of interest

None declared.

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