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Investigation into the vasospastic mechanisms in the pathogenesis of glaucomatous neuropathy

Badanie udziału mechanizmów naczynioskurczowych w patogenezie neuropatii jaskrowej

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Summary: Spasm of blood vessels supplying the optic nerve head is considered one of possible ischaemic mechanisms of glaucomatous optic neuropathy.

Purpose: The aim of the study was to evaluate the role of two potent and long-acting vasoconstrictors: endothelin-1 (ET-1) and neuropetide Y (NPY) in the pathogenesis of glaucoma by:

1) measurement of plasma ET-1 and NPY concentrations in primary open-angle glaucoma (POAG) patients with high intraocular pressure (HTG patients) and with normal intraocular pressure (NTG patients) at baseline and following peripheral exposure to cold (cold-pressor test),
2) assessment whether changes, if any, in the plasma concentrations of both peptides following the cold-pressor test correlate with visual field defects.

Material and methods: The study was conducted in three groups of subjects: 1) HTG patients, 2) NTG patients and 3) controls. All subjects were young and free from any cardiovascular disorders. ET-1 and NPY concentrations in the plasma were measured by radioimmunoassay (ET-1: Amersham International UK, NPY: Peninsula Laboratories INC). The cold-pressor test was performed by immersing the whole hand in ice-cold water (4°C) for 2 minutes. Visual fields were examined using standard automated perimetry (Octopus 101, G-2 programme, normal strategy).

Results: In the NTG patients the mean baseline plasma ET-1 concentration was significantly lower and the mean baseline plasma NPY concentration significantly higher compared to controls. On the other hand, there were no statistically significant differences in the mean baseline peptide levels between the HTG patients and the control subjects. After the cold-pressor test the mean ET-1 concentrations considerably increased in the three groups. The highest increase was seen in the NTG group and it was statistically significant compared to the HTG group and controls. Following the cold-pressor test the mean NPY concentration was significantly decreased in the NTG group, but remained virtually unchanged in the HTG group and controls. In the NTG patients, significant increase in the mean ET-1 concentration and decrease in the mean NPY concentration seen after the cold-pressor test were accompanied by a significant decrease in the mean MS (mean retinal sensitivity) value in the second eye examined after the cold-pressor test, but no correlation was found between changes in the MS values and changes in the ET-1 and NPY concentrations. There were no significant changes in the mean MS values after cold-pressor test in the HTG patients and controls.

Conclusions: Our findings suggest abnormal neuro-endothelial mechanisms of vascular tone control in NTG patients, related to the effects of ET-1 and NPY, secondary to endothelial dysfunction and to dysregulation of the autonomic nervous system. These abnormalities may involve potentiation of the vasoconstrictive effects of both ET-1 and NPY leading to the optic nerve head ischaemia and subsequent development of visual field defects in the course of normal-tension glaucoma.

Key words: Glaucoma, vasospasm, endothelin-1, neuropetide Y, visual field, cold-pressor test.

Streszczenie: Skurcz naczyń krwionośnych, zaopatrujących głowę nerwu wzrokowego, uważany jest za jeden z możliwych patomechanizmów niedokrwiennych neuropatii jaskrowej.

Cel: celem pracy jest próba oceny roli dwóch silnie i długo działających mediatorów naczynioskurczowych – endoteliny-1 (ET-1) i neuropeptydu Y (NPY) – w patogenezie jaskry poprzez:

1) określenie stężeń tych peptydów we krwi u chorych na jaskrę pierwotną otwartego kąta (JPOK) z ciśnieniem śródgałkowym – zarówno wysokim (JWC), jak i normalnym (JNC) – w warunkach podstawowych i po obwodowej ekspozycji na działanie zimna (test ochłodzenia),
2) ocenę, czy ewentualne zmiany w stężeniu peptydów we krwi po teście ochłodzenia są skorelowane ze zmianami w polu widzenia.

Materiał i metody: badanie przeprowadzono w trzech grupach: 1) chorych na JWC, 2) chorych na JNC, 3) kontrolnej. Do badania zakwalifikowano osoby młode, które nie były obciążone chorobami układu krążenia. Pomiary stężeń ET-1 i NPY zostały wykonane metodą radioimmunologiczną (ET-1: Amersham International UK, NPY: Peninsula Laboratories INC). Test ochłodzenia polegał na zanurzeniu dłoni w kąpieli wodnej o temperaturze 4°C przez 2 min. Pole widzenia badano metodą standardowej statycznej perymetrii komputerowej (Octopus 101, program G-2, strategia normalna).

Wyniki: u chorych na JNC średnie stężenie ET-1 we krwi w warunkach podstawowych było istotnie niższe, a NPY – istotnie wyższe, wg porównania z grupą kontrolną. Nie stwierdzono statystycznie istotnych różnic w zakresie podstawowych średnich stężeń peptydów, wg porównania grupy chorych na JWC z grupą kontrolną. Po teście ochłodzenia wystąpił znaczący wzrost średniego stężenia ET-1 u osób we wszystkich trzech grupach, najwyższy w grupie chorych na JNC i istotny statystycznie wg porównania grup JWC i kontrolnej. Średnie stężenie NPY po teście ochłodzenia uległo istotnemu obniżeniu u chorych na JNC, natomiast nie uległo istotnym zmianom u pacjentów w pozostałych dwóch grupach. Znaczącemu wzrostowi średniego stężenia ET-1 i spadkowi średniego stężenia NPY po teście ochłodzenia u chorych na JNC towarzyszył istotny spadek średniej wartości parametru MS (średnia czułość siatkówki) w polu widzenia oka, które badano w drugiej kolejności po teście ochłodzenia, nie stwierdzono jednak istotnej korelacji między zmianami wartości MS a zmianami stężeń ET-1 i NPY. Nie stwierdzono istotnych zmian średniej wartości parametru MS po teście ochłodzenia u pacjentów w grupach JWC i kontrolnej.
Wnioski: wyniki prezentowanej pracy przemawiają za istnieniem nieprawidłowych mechanizmów nerwowo-śródbłonkowej kontroli napięcia ściany naczyniowej u pacjentów z jaskrą normalnego ciśnienia, związanych z działaniem ET-1-1 i NPY, o charakterze dysregulacji układu współczulnego i dysfunkcji śródbłonka. Zaburzenia te mogą być związane z nasileniem naczynioskurczowego działania obu peptydów i mogą być jednym z czynników, które prowadzą do niedokrwienia nerwu wzrokowego oraz powstawania zmian w polu widzenia w przebiegu jaskry normalnego ciśnienia.
Słowa kluczowe: jaskra, skurcz naczyniowy, endotelina-1, neuropeptyd Y, pole widzenia, test ochłodzenia.

A possible pathogenetic association between vasospasm with disordered autoregulation of blood flow in the optic nerve head and the development of glaucomatous optic neuropathy was first suggested in the late nineties (1,2). According to the present knowledge of neuroendothelial control of vascular tone, vasospasm may be produced by the activity of the sympathetic nervous system or result from the predominance of the vasoconstrictive endothelial factors over the vasodilative factors (endothelial dysfunction). Neuropetide Y (NPY), is a neurotransmitter found in the sympathetic nervous system which is responsible for a powerful and long-lasting vasospasm. Endothelin-1 (ET-1), a peptide derived from endothelial cells is the most potent known vasoconstrictor. The arguments for the possible role of these two neurotransmitters in the pathogenesis of glacomatous optic neuropathy are presented in an earlier article by the present authors (3).

The aim of the study was to evaluate the role of ET-1 and NPY in the pathogenesis of glaucomatous neuropathy by: 1) measurement of plasma ET-1 and NPY concentrations in primary open-angle glaucoma (POAG) patients with high intraocular pressure (high-tension glaucoma, HTG), and with normal intraocular pressure (normal-tension glaucoma, NTG), at baseline and following peripheral exposure to cold (cold-pressor test); 2) assessment whether changes, if any, in the plasma levels of both peptides following this test correlate with visual field defects.

Patients and methods

The study was approved by the Ethics Committee at the Medical Centre of Postgraduate Education and the subjects gave their written informed consent.

17 patients with POAG were included in the study, subdivided into two study groups in the same age range: 9 HTG patients: 6 females and 3 males, at the age of 20-47 years (mean age 33) and 8 NTG patients: 7 females and 1 male, at the age of 25-45 years (mean age 36). Gonioscopy was performed in all patients to exclude congenital abnormalities. A control group consisted of 16 healthy non-glacomatous volunteers: 9 females and 7 males, at the age of 28-45 years (mean age 34 years), who were age-matched (mean ages 33 and 35 years of females and males respectively), without ocular and systemic diseases or a history of tendency to peripheral vasospasm. The study patients had slightly or moderately advanced glaucoma (C/D$<$0.8). Examination of the visual fields was performed by standard automated computed perimetry (Octopus 101, G-2, normal strategy). The perimetry finding was considered abnormal if typical glaucomatous defects according to the "Terminology and Guidelines for Glaucoma" classification were identified. In the cases of preperimetric glaucoma, the diagnosis was additionally supported by short-wave perimetry (blue-on-yellow perimetry), assessment of the optic nerve disc by scanning laser tomography (Heidelberg Retina Tomograph, HRT), or measurement of the retinal nerve fibre layer thickness by scanning laser polarimetry (GDx). All patients had been treated with topical drugs which were discontinued 2-3 weeks before the study or had remained untreated after previous glaucoma surgery.

As blood ET-1 levels may rise with advancing age and in cardiovascular diseases due to endotelial dysfunction, young individuals without such diseases and non-smokers were enrolled in the study. Premenopausal and menopausal women (upper age limit of 47 years), and obese subjects were not included because of potential effects of hormonal changes and obesity leading to elevated NPY concentrations in the blood. The upper age limit for males was adjusted to the age limit for females. Since gender-dependent differences in blood concentrations of ET-1 and NPY could not be excluded, before the mixed-gender study groups were formed, these concentrations had been compared at baseline and after the cold-pressor test in females and males from the control group.

The cold-pressor test was performed by immersing the whole hand in ice-cold water (4°C) for 2 minutes. ET-1 and NPY concentrations were measured by radioimmunoassay (ET-1: Amersham International UK, NPY: Peninsula Laboratoties INC). In part 1 of the study performed in subjects from the three groups: HTG, NTG and controls, ET-1 and NPY concentrations were measured in blood samples drawn from the antecubital vein in the morning (baseline measurements under physiological conditions), and the visual fields were examined. In part 2 of the study (2 hours later), ET-1 and NPY concentrations were measured in venous blood immediately after the cold-pressor test (performed on the other hand), and the visual fields were re-examined. The evaluations included differences in the mean concentrations of ET-1 and NPY between groups at baseline and changes in their mean concentrations after the cold-pressor test. The visual field examinations were performed

 ISSN 0023-2157 Index 362646

in all glaucoma patients and in 11 controls (8 females and 3 males at the age of 28-45 years, mean age 34 years), in whom a reliable computerized examination of the visual fields was feasible. The sequence at which the eyes were examined after the cold-pressor test was taken into consideration. Examination of the visual field in one eye takes approximately 10-15 minutes, which means that the time from exposure to cold and onset of possible vasospastic response is different for the eye examined immediately after the cold-pressor test (Eye-1) and the second examined eye (Eye-2). Thus the haemodynamic conditions in the area of blood supply to the optic nerve may differ with different effects on its function affecting the ultimate perimetry findings. Changes in the mean MS (mean retinal sensitivity) value of computerized perimetry after the cold-pressor test were evaluated. Also assessed were correlations between changes in the ET-1 and NPY concentrations and changes in the MS values in the three groups. Statistical analysis was performed with the Kolmogorov-Smirnov tests, Student-*t* test for dependent and independent variables and Pearson correlation coefficients at $p<0.05$.

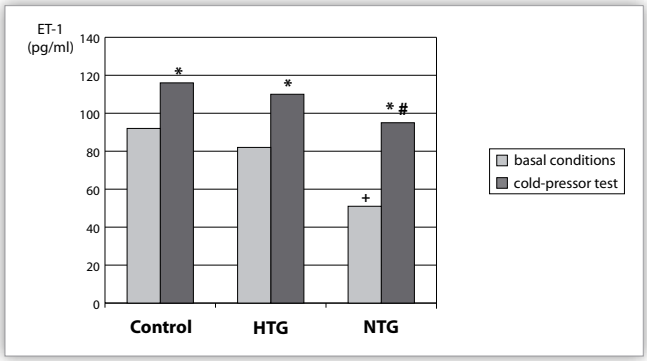

Fig. 1. Mean plasma ET-1 concentrations in basal conditions and after cold-pressor test in the Control, HTG and NTG groups
\+ $p<0,05$ in comparison to the Control and HTG groups
* $p<0,05$ in comparison to basal conditions
\# $p<0,05$ in comparison to mean plasma ET-1 concentration increase after cold-pressor test in the Control group

Ryc. 6. Średnie stężenia ET-1 w surowicy w warunkach podstawowych i po teście ochłodzenia u pacjentów w grupach kontrolnej, JWC i JNC.

Results

1. Mean blood concentrations of ET-1 and NPY at baseline did not differ significantly between non-glacomatous female and male volunteers (ET-1: 90.28 ± 7.12 vs 92.50 ± 6.77 pg/ml; NPY: 10.72 ± 2.04 vs 10.79 ± 1.66 pg/ml). After the cold-pressor test the mean blood ET-1 concentrations significantly increased in non-glacomatous females and males by 25.22 ± 18.65 pg/ml and 24.64 ± 15.76 pg/ml respectively (no significant difference in the increase between females and males). After the cold-pressor test the mean NPY concentration did not changed significantly: it decreased by 0.33 ± 2.38 pg/ml in females and increased by 0.64 ± 1.80 pg/ml in males. As there were no significant differences between non-glacomatous females and males in the mean concentrations of both ET-1 and NPY at baseline and in their changes following the cold-pressor test it was assumed that in this age group, the gender factor had no effect on the physiological and cold-induced release of both peptides, which allowed formation of mix-gender study groups.
2. Mean blood ET-1 concentrations at baseline did not significantly differ between the controls and the HTG patients, but it was significantly lower in the NTG patients compared to the other two groups (controls: 91.25 ± 6.77, HTG: 81.39 ± 27.36, NTG: 50.63 ± 24.34 pg/ml). After the cold-pressor test, in the three groups there was a statistically significant increase in mean ET-1 concentrations compared to baseline. The increase was highest in the NTG group, being significant compared to controls (controls: +24.97 ± 17.11, HTG: +28.06 ± 21.64, NTG: +43.75 ± 19.27 pg/ml) (Tab. I, Fig. 1).
3. At baseline, the highest mean blood concentration of NPY was found in the NTG group and the difference was statistically significant compared to controls and at the borderline of statistical significance compared to the HTG patients

Group	ET – part 1	ET – part 2	Δ ET
Control	**91,25** + 6,77	**116,22** + 16,21	**+24,97** + 17,11
HTG	**81,39** + 27,36	**109,44** + 19,28	**+28,06** + 21,64
NTG	**50,63** + 24,34	**94,38** + 11,86	**+43,75** + 19,27

Tab. I. Mean plasma ET-1 concentrations (pg/ml) (± SD) in the Control, HTG and NTG groups in basal conditions (part 1) and after cold-pressor test (part 2).

Tab. I. Średnie stężenia ET-1 w surowicy (pg/ml) (± SD) u pacjentów w grupach kontrolnej, JWC i JNC w warunkach podstawowych (część 1.) i po teście ochłodzenia (część 2.).

Group	NPY – part 1	NPY – part 2	Δ NPY
Control	**10,75** + 1,66	**10,84** + 1,41	**+ 0,09** + 2,14
HTG	**11,50** + 1,46	**11,67** + 1,58	**+ 0,17** + 1,00
NTG	**13,38** + 2,67	**10,44** + 1,80	**- 2,94** + 2,43

Tab. II. Mean plasma NPY concentrations (pg/ml) (± SD) in the Control, HTG and NTG groups in basal conditions (part 1) and after cold-pressor test (part 2).

Tab. II. Średnie stężenia NPY w surowicy (pg/ml) (± SD) u pacjentów w grupach kontrolnej, JWC i JNC w warunkach podstawowych (część 1.) i po teście ochłodzenia (część 2.).

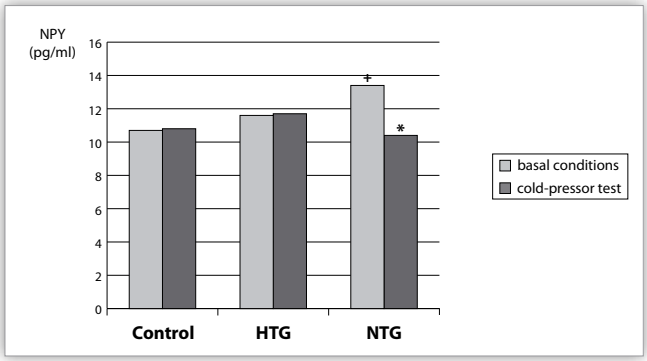

Fig. 2. Mean plasma NPY concentrations in basal conditions and after cold-pressor test in the Control, HTG and NTG groups
\+ $p<0,05$ in comparison to the control group
* $p<0,05$ in comparison to basal conditions

Ryc. 2. Średnie stężenia NPY w surowicy w warunkach podstawowych i po teście ochłodzenia u pacjentów w grupach kontrolnej, JWC i JNC.

($p = 0,051$) (controls: 10.75 ± 1.66, HTG: 11.50 ± 1.46, NTG: 13.88 ± 2.67 pg/ml). After the cold-pressor test there were no significant differences compared to baseline in the mean NPY concentrations in controls and the HTG patients, but there was a statistically significant decrease in the mean NPY concentration in the NTG patients (controls: +0.09 ± 2.14, HTG: +0.17 ± 1.00, NTG: -2.94 ± 2.43 pg/ml) (Tab. II, Fig. 2).

4. In the control group, after the cold-pressor test the mean MS value increased in the eye examined immediately after the test (Eye-1) (+0.31 ± 0.47 dB), and decreased in the second examined eye (Eye-2) (-0.09 ± 0.65 dB). In both cases the differences were not statistically significant. Following the test, in the HTG and NTG patients, the mean MS values decreased in the eye examined immediately after the test (HTG: -0.53 ± 1.37 dB, NTG: -0.5 ± 1.17 dB), but the reduction was not statistically significant. In the second examined eye, the mean MS value was decreased to a lesser extent in the HTG group (-0.27 ± 1.08 dB, no statistically significant difference), while in the NTG group there was a significant decrease in the mean MS value compared to baseline (-1.06 ± 1.04 dB) (Tab. III, Fig. 3).

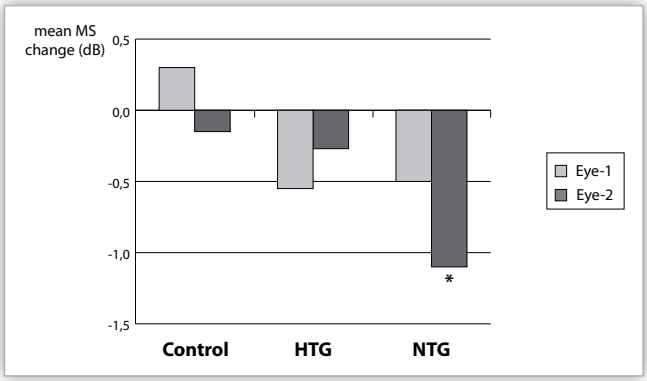

Fig. 3. Changes of mean MS value (dB) in the visual fields in Control, HTG and NTG groups in the eye examined immediately after cold-pressor test (Eye-1) and in the second examined eye (Eye-2).
* $p < 0,05$ in comparison to basal conditions

Ryc. 3. Zmiany średniej wartości parametru MS (dB) w polu widzenia u pacjentów w grupach kontrolnej, JWC i JNC w oku badanym bezpośrednio po teście ochłodzenia (Oko-1) i w oku badanym w drugiej kolejności po teście (Oko-2).

5. In the NTG group, a significant decrease in the mean MS value in the second examined eye was accompanied by significant increase in the mean ET-1 concentration, compared to baseline and to controls, and a statistically significant decrease in the mean NPY concentration. However, there was no correlation between changes in the concentrations of both peptides and changes in MS values in this eye (ET-1: $r = 0.429$; NPY: $r = 0.512$).

Discussion

To date, there have been no published studies on NPY measurements in patients with POAG and only a few reports of blood ET-1 concentrations in these patients (4-9). In our study the mean blood NPY concentration was higher in the NTG group than in the other two groups and the difference was statistically significant compared to controls. Most studies which measured ET-1 blood concentrations in NTG patients (4,5,7) found higher levels than in non-glacomatous individuals. Those findings disagree with the results of our study where the mean ET-1 blood concentration was significantly lower compared to controls and HTG patients. Differences in subjects' age may to a certain extent account for this difference. Previous studies were performed in older glaucoma patients (mean age about 60 years), while the mean age of NTG and HTG patients in this study were 33 and 36 years. Age and co-existing cardiovascular diseases are closely associated with progressing endothelial dysfunction and increasing ET-1 blood levels. Elevated ET-1 levels in NTG patients may be due to more frequent, compared to the general population, co-existence of NTG and cardiovascular disease.

The clinical picture of NTG suggests that the pathogenesis may be different in older and younger individuals. In older patients, damage to the optic nerve disc is usually "atrophic" and associated with cardiovascular diseases while in younger patients the "focal" type is common with low systemic blood pressure, migraine and tendency to vasospasm. These observations were taken into consideration in the criteria of inclusion into the present study and enrollment of younger glaucoma patients in search for possible primary endothelial dysfunction. The age limit was also suggested by the observation that a tendency to vasospasm decreases with age (such tendency is characteristic for younger women, and often disappears after menopause-1). The study groups were small in size because POAG is relatively rare in younger individuals.

Group	Eye-1			Eye-2		
	MS-part 1	MS-part 2	Δ MS	MS-part 1	MS-part 2	Δ MS
Control	**28,43** +1,21	**28,74** +1,01	**+0,31** +0,47	**28,65** +1,02	**28,55** +1,12	**-0,09** +0,65
HTG	**25,03** +2,89	**24,50** +3,68	**-0,53** +1,37	**24,54** +4,02	**24,28** + 4,14	**-0,27** +1,08
NTG	**26,69** +1,51	**26,19** +1,52	**-0,50** +1,17	**26,74** +1,29	**25,68** +1,75	**-1,06** +1,04

Tab. III. Mean MS values (dB) (± SD) in the computerised visual fields in Control, HTG, NTG groups in basal conditions (part 1) and after cold-pressor test (part 2) in the eye examined immediately after the cold-pressor test (Eye-1) and the second examined eye (Eye-2).

Tab. III. Średnie wartości parametru MS (dB) (± SD) w badanym komputerowo polu widzenia u pacjentów w grupach kontrolnej, JWC, JNC w warunkach podstawowych (część 1.) i po teście ochłodzenia (część 2.) w oku badanym bezpośrednio po teście (Oko-1) i w oku badanym w drugiej kolejności po teście (Oko-2).

ISSN 0023-2157 Index 362646

Both increased ET-1 concentration in older NTG patients and its lower level in younger NTG patients found in the present study demonstrate abnormal endothelium arrangement in patients with this form of POAG. The pathogenesis of the condition may be more complex and depend on other factors than endothethelial dysfunction related to age and cardiovascular comorbidities.

Significantly lower mean blood ET-1 level and higher NPY level in younger NTG patients compared to non-glacomatous subjects suggest an abnormal neuro-endothelial control of vascular tone in these patients. A significantly higher mean blood NPY concentration in NTG patients suggests neurogenic spasm of blood vessels. It seems, however, that elevated NPY levels in NTG patients may be a manifestation of sympathetic dysregulation rather, because under physiological conditions NPY modulates adrenergic effects. At the postsynaptic level, the neuromodulating effect of NPY consists of potentiating the vasoconstrictive activity of noradrenalin (NA), while at the presynaptic level it inhibits NA release. A significantly higher mean NPY level in the NTG group suggests dysregulation of the sympathetic system, i.e. inhibition of NA release from the sympathetic nerve endings with possible postsynaptic potentiation of NA vasoconstrictive activity.

Studies by other authors also suggest some dysregulation of the sympathetic nervous system in normal tension glaucoma (5). After changing from the supine to the upright position, blood ET-1 concentrations decreased in NTG patients, but significantly increased in non-glacomatous subjects and in HTG patients (5). Since postural changes did not produce any significant differences in the heart rate between the NTG patients and controls, it may be concluded that a similar activation of the sympathetic system produced different effect on ET-1 concentration in the blood. In NTG patients the effect might be due to the activation by NA of the α-2 receptor alone with the resulting inhibition of ET-1 production. This may suggest a selective effect of NA on the α-2 receptor and allow the hypothesis that NA binding to the major vasoconstrictive receptor α-1 may be impaired in NTG patients. A compensatory increase in the sympathetic activity and increased secretion of NA and NPY with subsequent overstimulation of the α-2 receptor and decreased blood ET-1 concentration could occur as a result. Such a mechanism may explain high NPY and low ET-1 levels in the NTG patients found at baseline in our study. This hypothesis may also account for reduced vasoconstrictive effect of sympathetic nervous system in NTG patients which can be related to systemic hypotension commonly seen in these patients. Weaker binding of NA to the α-1 receptor in NTG patients is suggested by abnormalities of orthostatic hypotension which is often associated with a tendency to peripheral vasospasm and is significantly (three-fold) more frequent in NTG patients compared to HTG patients (10). A defect in NA binding to the α-1 receptor may occur in orthostatic hypotension which may account for the co-existence of low arterial blood pressure and weakened baroreflex effect together with elevated NA blood level (11). In patients with orthostatic hypotension similarly to NTG patients, the response to postural change is abnormal, i.e. blood ET-1 level does not increase (12), which also may indicate common pathogenetic mechanisms in both diseases. If the defect in the NA binding to the α-1 receptor does occur in NTG patients, it may explain the neuromodulating effect of elevated NPY level in these patients: postsynaptic potentiation of the weakened vasoconstrictive response and presynaptic reduction of NA release, which may be increased as a possible compensatory mechanism.

Just as the sympathetic system affects the release of endothelial neurotransmitters, the endothelium may modulate the adrenergic effects which may account for low blood ET-1 levels in NTG patients seen in the present study. High ET-1 concentration enhances NA-induced vasospasm by stimulation of the postsynaptic ET-A receptors while its low concentration impedes the vasoconstrictive activity of NA by the presynaptic inhibition of its release (ET-B and ET-A receptors). As under physiological conditions ET-1 concentration in the blood is very low, its most important activity may be anti-adrenergic. If such is the case, the lowest baseline ET-1 levels in our NTG patients may be an argument in favour of the inhibitory effect on excessive NA release.

The cold-pressor test in the present study caused a significant increase in the mean blood ET-1 concentrations in the three groups, which shows that this physiological response is preserved also in glaucoma patients. Activation of the sympathetic system during the test produces constriction of blood vessels and an increase in the shear stress (the viscous drag exerted by the circulating blood), which is a very powerful factor stimulating ET-1 synthesis. The finding indicates that ET-1 may be involved in cold-induced vasospasm. Also other authors have described increased ET-1 concentrations following the cold-pressor test in healthy individuals and patients with cardiovascular diseases. In one study performed, patients with POAG did not show any changes in blood ET-1 concentrations following the cold-pressor test, which might have been due to a short period of exposure to cold (30 seconds) (13), but in another study in POAG patients, the ET-1 concentrations did significantly increase after the test (8). In our study, the highest mean ET-1 increase after the cold-pressor test was observed in the NTG group: it was nearly twice as high as in controls (statistical significance) and 1.5-times higher than in the HTG patients. As the vasoconstrictive effect of ET-1 increases with concentration, the finding indicates that cold-induced vasospasm is the most powerful in NTG patients. Cooling stimulates endothelial nitric oxide (NO) production, which inhibits ET-1 synthesis. Accordingly impaired NO synthesis may be a possible cause of the excessive increase in ET-1 concentrations observed in NTG patients following cooling. The attenuated vasodilatatory response to intravenous acetylcholine seen in NTG patients suggests that endothelial dysfunction related to diminished NO release may be responsible for the exaggerated vasoreactivity in NTG (14). The same authors suggest impaired ET-B receptor-related NO release in these patients (9). Elevated baseline ET-1 levels observed in older NTG patients indicate persistent endothelial dysfunction while in younger patients such dysfunction may be manifested only when the demand for NO is increased (NO release in response to cold impedes excessive vasoconstriction).

ET-1 enhances the vasoconstrictive effect of NPY while both peptides, in a concentration-dependent manner potentiate the vasoconstrictive effect of the sympathetic stimulation, which is more obvious at low temperature. Elevated baseline

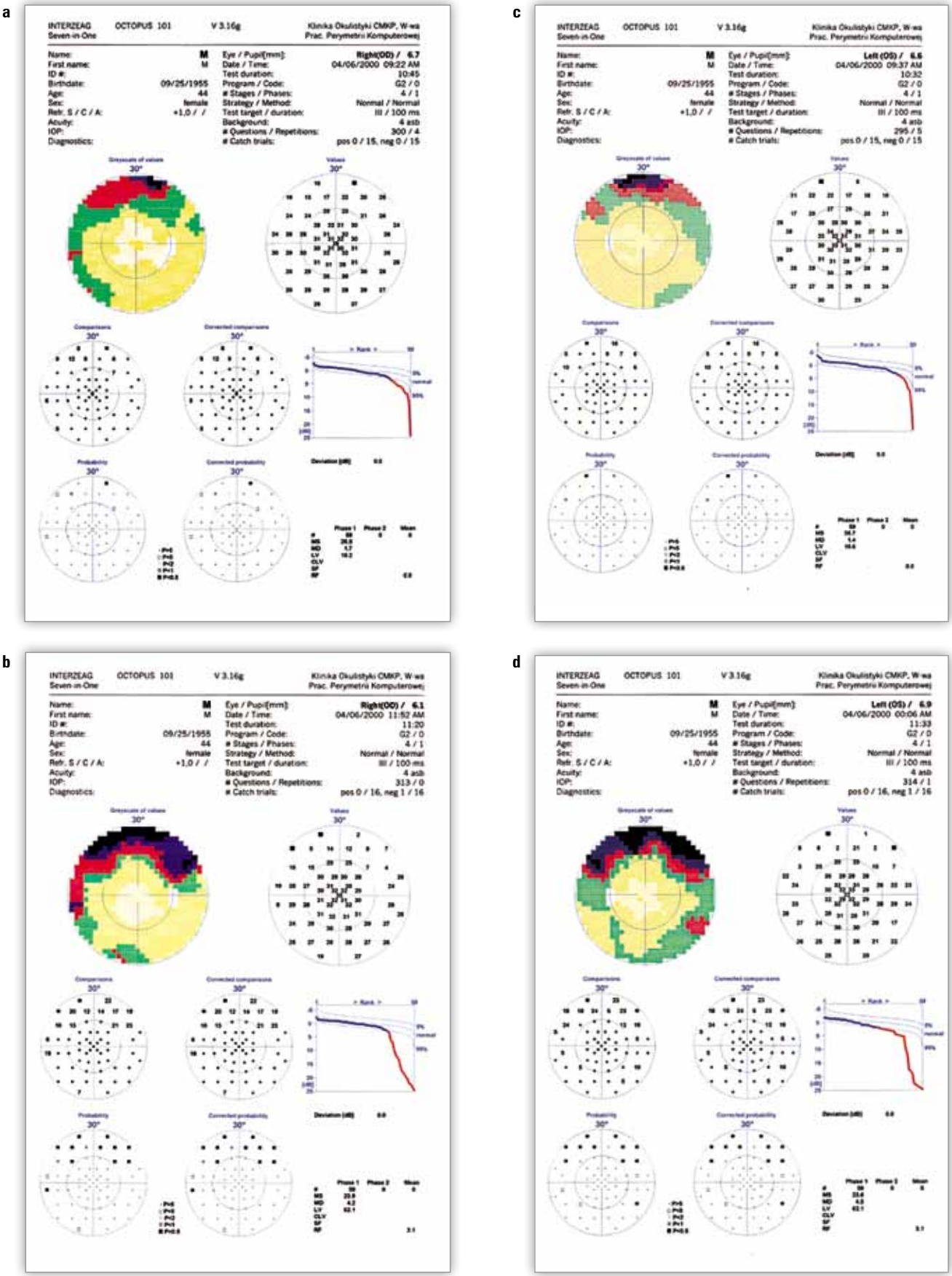

Fig. 4. Example of visual field changes after cold-pressor test in a patient from NTG group.
a. OD – before cold-pressor test; b. OD – after cold-pressor test; c. OS – before cold-pressor test; d. OS – after cold-pressor test

Ryc. 4. Przykład zmian w polu widzenia po teście ochłodzenia u pacjenta z grupy JNC.
a. OD – przed testem ochłodzenia; b. OD – po teście ochłodzenia; c. OS – przed testem ochłodzenia; d. OS – po teście ochłodzenia

ISSN 0023-2157 Index 362646

NPY level and the highest increase of ET-1 concentration after cold-pressor test in NTG patients suggest that in such patients cold-induced vasoconstriction may be signifiantly more powerful than in healthy individuals and HTG patients.

In the present study, the cold-pressor test did not produce any significant changes in the mean blood NPY concentrations in the non-glaucomatous volunteers and in the HTG patients while in the NTG patients there was a significant fall in the mean NPY level, which was quite unexpected considering that the cold-pressor test stimulates the sympathetic system. Since the NPY-ergic innervation becomes reduced with decreasing diameter of the arterial blood vessels, cold-induced spasm of smaller arteries and microcirculation may cause slower NPY flow into the veins. NPY release into the venous circulation in response to exposure of the hand to cold was delayed in healthy individuals (15). This may to a certain extent account for the absence of changes in NPY levels in the venous blood of controls and HTG patients following the cold-pressor test in our study. Presumably, in the NTG group the cold-induced rise in ET-1 concentration, which was higher than in controls or HTG patients, combined with high baseline NPY levels, triggered a more powerful vasopam of the arterioles and microcirculation, which may have resulted in a more marked delay of NPY release into the veins. The best explanation for the decreases in mean NPY level after cold-pressor test in our NTG group seems to be inhibition of NPY release by ET-1 (16), which should be most prominent in NTG patients, leading to the fall of NPY blood concentration.

The results of the present study indicate the presence of complex disturbances of the neuroendothelial control of vascular tone in NTG patients seen as sympathetic dysregulation and endothelial dysfunction, which may lead to potentiation of vasospasm in response to cold. In HTG patients, the mean baseline blood ET-1 and NPY concentrations did not significantly differ from the values in the non-glacomatous subjects which is in agreement with the results reported by other authors (6,8). Also, changes in the mean levels of both peptides induced by cold were not significantly different in HTG patients compared to controls. These findings indicate that disorders of the neuroendothelial control of vascular tone are not characteristic of HTG patients.

The cold-pressor test used in glaucoma to evaluate changes in the visual fields induced by cold has demonstrated a particular tendency to reduced retinal sensitivity in NTG patients (2,17). Also the present study found a significant decrease in the mean MS value in the second examined eye (Eye-2). In our NTG group the lowest decrease in the MS value for both eyes (by -2.6 and -3.1 dB) was seen in a female patient with a family history of Raynaud's disease (Fig. 4).

A significant reduction of the mean MS value in the second examined eye (Eye-2) in the NTG patients was accompanied by a significant increase in the mean blood ET-1 concentration, which may suggest that peptide-induced vasoreactivity is the underlying cause of the visual field changes. However, there was no significant correlation between changes in ET-1 concentrations and MS changes in the NTG patients, which may have been due to the small size of the study groups.

The hand cold-pressor test, by systemic activation of the sympathetic system, causes increases in ET-1 concentrations in other vascular beds of the body. Since the choroid and extraocular vessels are supplied by the sympathetic nerves, tonus of the sympathetic system also in this part of the optic nerve supply is likely to increase with subsequent ET-1 release. The highest, statistically significant increase in the mean blood ET-1 concentration observed after the cold-pressor test in the NTG patients in our study combined with the significantly higher mean NPY level at baseline was likely to cause stronger spasm of the peripapillary choroid and extraocular vessels upon exposure to cold. Spasm of these vessels which supply the optic nerve head in its prelaminar and laminar parts may have led to a decrease in the perfusion pressure in this area and triggered autoregulary vascular dilatation, which caused partial depletion of the autoregulatory reserve, higher in NTG patients. ET-1 may penetrate through the fenestrated capillaries of the peripapillary choroid into the optic nerve head and exert a vasoconstrictive effect in this area, which may have caused inhibition of the autoregulatory vasodilator response, also more potent in NTG patients. One may not exclude involvement of endothelial dysfunction in the pathomechanism of autoregulation disturbances, which is characteristic for NTG patients. As a result of these conditions the autoregulatory mechanisms in the optic nerve of NTG patients may have been exhausted causing ischaemia of its fibres clinically manifested as a significant decrease in retinal sensitivity. The visual field defect following exposure to cold in the eye examined 15 minutes after the exposure (Eye-2) indicates that ischaemia secondary to vasospasm must be present for some time before dysfunction of the optic nerve fibres occurs. Dynamics of changes in the visual fields of both eyes suggests that the initial ischaemia of the nerve fibres, which caused a non-significant decrease in the retinal sensitivity in the eye examined immediately after the test (Eye-1) did not trigger a sufficient vasodilatative response capable of restoring adequate blood flow and preventing further deterioration of the visual field in the other eye (Eye-2). This may also indicate weakened endothelial mechanisms responsible for metabolic autoregulation in NTG patients.

The results of the present study indicate that abnormal neuro-endothelial mechanisms of vascular tone control may be found in NTG patients, related to the effects of ET-1 and NPY and due to endothelial dysfunction and dysregulation of the autonomic nervous system. These abnormalities may involve potentiation of the vasoconstrictive effects of both ET-1 and NPY leading to the optic nerve head ischaemia and subsequent development of visual field defects in the course of normal-tension glaucoma.

This study was supported by a grant number 501-2-2-14-22/99 from Medical Centre of Postgraduate Education, Warsaw, Poland.

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List of remaining literature references available on request from the authors.

The study was originally received 17. 04.2011 (1301)/
Praca wpłynęła do Redakcji 17. 04.2011 r. (1301)
Accepted for publication 14.07.2011/
Zakwalifikowano do druku 14.07.2011 r.

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ISSN 0023-2157 Index 362646