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Anterior Pituitary Weight, cAMP, cGMP and Prolactin Levels After Combined Treatment with Estradiol and Methylene Blue

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Summary

Male rats received estradiol benzoate in a long acting microcrystalline suspension (1 mg/rat i.m., twice a week), methylene blue (MB) 0.5 % in the food and the combination of estradiol and MB. After three weeks, MB partially inhibited the growth response of the anterior pituitary to estradiol and it partially inhibited the increase of cAMP content in anterior pituitary. The increase of anterior pituitary cGMP content was not modified by MB, neither the ratio cAMP/cGMP in the anterior pituitary which, however, decreased after estradiol. This decrease was not modified by MB. On the other hand, the prolactin (PRL) increase in the blood after estradiol was inhibited by MB, although the prolactin content in the anterior pituitary was not. Methylene blue alone did not change blood prolactin concentration, but it unexpectedly elevated blood thyroxine levels and this effect was partially inhibited by simultaneous estradiol treatment.

Key words

Estradiol – Anterior pituitary – Methylene blue – cAMP – cGMP – Prolactin

Introduction

Nitric oxide (NO), identical to endothelium-derived relaxing factor, is also formed in brain tissue (Bredt *et al.* 1990, Snyder 1992) and may participate in the damage of cells produced by superoxide free radicals. NO is an activator of cytosolic guanylate cyclase producing cGMP and this reaction can be inhibited by methylene blue (Ignarro 1989). In this way, methylene blue can inhibit some physiological reactions mediated by nonadrenergic-noncholinergic neurotransmission. Methylene blue can also inhibit some pathological reactions, e.g. exaggerated vasodilatation and hypotension in liver failure (Midgley *et al.* 1991).

Estrogen-induced adenohypophyseal growth response, first described by Selye (1944), is accompanied by increased cAMP and cGMP levels in the adenohypophysis (Nedvídková and Schreiber 1992), as well as by increased prolactin secretion and prolactin-cells nucleolar activation (Dušková and Schreiber 1989). These changes can be inhibited by an excess of thyroid hormones.

We therefore wondered, whether the anterior pituitary response to estradiol could be modified by simultaneous administration of methylene blue.

Material and Methods

In two identical experiments, 40 male rats (Wistar strain descendants, Velaz, Prague) kept at 22±2 °C in a 12 h light – 12 h darkness regimen and fed a standard laboratory diet (Larsen diet, Velaz, Prague) were divided into four groups: 1. Controls, 2. those administered estradiol benzoate in a microcrystalline suspension 1 mg/rat, twice a week, i.m. (Agofollin depot, SPOFA, Prague, EB), 3. those given 0.5 % methylene blue (Loba Feinchemie, Austria, MB) and 4. those receiving both estradiol + methylene blue (EM + MB).

After three weeks of treatment the animals were killed by decapitation, the blood was collected and the anterior pituitaries were isolated, weighed and quickly frozen in liquid nitrogen.

Anterior pituitary content of cAMP and cGMP was determined by radioimmunoassay kits (ADICO, Prague). The frozen anterior pituitaries were homogenized in a Braun-Potter-Elvehjem all-glass homogenizer (10 strokes, 1000 rpm) in water containing 4 mol EDTA/l to prevent enzymatic breakdown of cAMP and cGMP, followed by 3 min heating in a boiling water bath to coagulate the proteins and by 30 min centrifugation (2000 x *g* at 4 °C) in a Beckmann GPR centrifuge. The supernatant was collected and frozen at −30 °C until required for cAMP and cGMP assay.

The blood and anterior pituitary content of prolactin was determined by double antibody RIA using NIADDK kit (rat PRL NIADDK rPRL-I-5 for radioiodination; NIADDK-rPRL-RP-3 as the reference preparation and rPRL NIADDK-rPRL-S-8 antiserum; see Acknowledgement). Blood thyroxine was determined by RIA using the antibody of our own provenience (Nedvídková and Felt 1977).

Means and 95 % confidence intervals were computed and the significance of differences of the means was evaluated by the analysis of variance and the multiple range and multiple F-test (Duncan 1955).

Results

The results are given in Tables 1 and 2. Estradiol treatment increased anterior pituitary weight, which was partially inhibited by methylene blue. This inhibitory effect ocurred in both experiments but was statistically significant in Experiment 2 only. In agreement with our previous observations (Nedvídková and Schreiber 1992), estradiol treatment resulted in a significant increase in anterior pituitary cAMP and cGMP content. This increase was inhibited by methylene blue significantly only as far as the cAMP anterior pituitary content in Experiment 2 is concerned. Estrogen treatment decreased the anterior pituitary cAMP/cGMP ratio and this reduction was not modified by methylene blue treatment.

Blood prolactin levels rose after estradiol treatment and this rise was inhibited by methylene blue. The rise of prolactin content in the anterior pituitary after estradiol was not modified by methylene blue treatment. Methylene blue did not change blood prolactin levels by itself but it unexpectedly increased blood thyroxine levels in both experiments.

Table 1
The results of Experiment 1.

Group	1. Controls (n = 10)	2. Estradiol benzoate (n = 10)	3. Methylene blue (n = 10)	4. Estradiol benzoate + methylene blue (n = 10)
Body weight, g				
Initial	203 ± 7	202 ± 6	206 ± 13	203 ± 8
Final	347 ± 26 (2-4)	206 ± 11 (1,3,4)	285 ± 13(1,2,4)	193 ± 12(1-3)
Anterior pituitary				
mg	11.12 ± 1.07 (2,3)	15.51 ± 2.19 (1,3)	9.71 ± 1.18 (1,2,4)	13.10 ± 2.75 (3)
mg/kg	32.29 ± 3.67 (2,4)	75.11 ± 9.49 (1,3)	33.91 ± 3.42 (2,4)	67.37 ± 12.59 (1,3)
cAMP in the anterior pituitary (AP)				
pmol/mg	2.39 ± 0.34 (2,4)	4.05 ± 0.70 (1,3)	2.50 ± 0.64 (2,4)	3.75 ± 0.75 (1,3)
pmol/AP	27.02 ± 5.41 (2,4)	63.24 ± 17.55 (1,3)	23.47 ± 4.00 (2,4)	51.44 ± 19.94 (1,3)
cGMP in the anterior pituitary				
pmol/mg	0.40 ± 0.08 (2-4)	1.33 ± 0.45 (1,3)	0.63 ± 0.07 (1,2,4)	1.18 ± 0.31 (1,3)
pmol/AP	4.50 ± 0.80 (2-4)	18.09 ± 5.66 (1,3)	6.19 ± 1.02 (1,2,4)	12.84 ± 2.73 (1,3)
c AMP/cGMP (AP)	6.38 ± 1.65 (2-4)	3.52 ± 0.93 (1)	4.14 ± 1.43 (1)	3.41 ± 0.73 (1)
Thyroxine, blood				
nmol/l	82.40 ± 7.21 (2-4)	72.21 ± 7.07 (1,3,4)	132.63 ± 13.94 (1,2,4)	114.39 ± 16.25 (1-3)

Means ± 95 % confidence intervals. The numbers of groups with significantly different means (Duncan's test) are given in parentheses.

Table 2
The results of Experiment 2.

Group	1. Controls (n=10)	2. Estradiol benzoate (n=10)	3. Methylene blue (n=10)	4.Estradiol benzoate + methylene blue (n=9)
Body weight, g				
initial	179±2	183±3	181±3	177±6
final	277±11 (2-4)	185±8 (1,3,4)	218±23(1,2,4)	167±9(1-3)
Anterior pituitary				
mg	8.54±1.09 (2,4)	21.69±3.14 (1,3,4)	7.47±0.90 (2,4)	16.56±3.22 (1-3)
mg/kg	30.70±3.20 (2,4)	116.34±14.81 (1,3,4)	32.32±3.87 (2,4)	98.42±17.44 (1-3)
cAMP in the anterior pituitary (AP)				
pmol/mg	2.90±0.21 (3)	2.75±0.74 (3)	3.72±0.62 (1,2,4)	2.75±0.96 (3)
pmol/AP	25.42±3.33 (2,4)	60.14±21.01 (1,3,4)	27.48±4.00 (2)	45.19±17.84 (1,2)
cGMP in the anterior pituitary				
pmol/mg AP	0.56±0.13 (2,4)	0.88±0.23 (1)	0.65±0.22 (4)	0.90±0.23 (1,3)
pmol/AP	5.07±1.91 (2,4)	18.68±5.16 (1,3)	4.85±1.97 (2,4)	16.75±5.05 (1,3)
cAMP/cGMP (AP)	5.48±0.96 (2,4)	3.13±0.51 (1,3)	6.23±1.16(2,4)	3.33±1.32 (1,3)
Thyroxine, blood				
nmol/l	90.38±6.80(2,3)	77.37±6.36 (1,3,4)	107.80±10.74 (1,2,4)	99.27±9.41(2,3)
Prolactin, blood				
ng/ml	4.8±1.8 (2,4)	94.0±37.05 (1,3)	5.37±2.80 (2,4)	70.1±13.12 (1,3)
Prolactin in the anterior pituitary				
ng/mg	0.35±0.20	0.34±0.07	0.40±0.04	0.44±0.19
ng/AP	2.84±1.23 (2,4)	7.26±1.56 (1,3)	2.95±0.37 (2,4)	7.28±2.88 (1,3)

Means ±95 confidence intervals. The numbers of groups with significantly different means (Duncan's test) are given in parentheses

Discussion

The experimental search for a pharmacological blocker of estrogen-induced anterior pituitary growth (which could be of clinical relevance in the treatment of prolactinomas) was not successful in this series of experiments. In previous experiments, we observed an inhibitory effect of thyroid hormones, testosterone, antiestrogens, dopaminergic agonists (lisuride), as well as a potentiating effect of dopaminergic antagonists (perphenazine) on estrogen-induced adenohypophyseal hypertrophy (Schreiber and Přibyl 1972).

Methylene blue may represent a new class of antioxidant drugs that competitively inhibit the reduction of molecular oxygen to superoxide by acting as alternative electron acceptors for tissue oxidases (Salaris *et al.* 1991). It thus could play a role in multiple transduction mechanisms of dopamine (Enjalbert 1989) in anterior pituitary cells. The question arises whether nitrergic mechanisms are involved in this complex interplay.

Methylene blue also inhibits some physiological and pathological reactions by its blocking action on cytosolic guanylate cyclase and cGMP production (stimulated by nitric oxide, Ignarro 1989). In our present experiments, however, it only partially interfered with the adenohypophyseal growth response to estrogen, as well as with hormonal (prolactin) reaction, occurring after estradiol. On the other hand,

the partial inhibitory effect of methylene blue can be explained by the unexpected increase of blood thyroxine levels after methylene blue treatment. Thyroid hormones were found, to inhibit the anterior pituitary weight response to estrogen (Schreiber and Přibyl 1972). The mechanism, by which methylene blue increased blood thyroxine levels remains to be elucidated.

Anyway, a nitrergic mechanism (involving the cascade NO synthase - NO - guanylate cyclase - cGMP) is probably involved in the anterior pituitary response to estradiol (Nedvídková and Schreiber 1992). Furthermore, in both present experiments, estradiol treatment increased cGMP content in the anterior pituitary, both when expressed per mg of the anterior pituitary or per whole gland. Therefore, the cAMP/cGMP ratio in the anterior pituitary decreased after estradiol treatment in both experiments. We know, of course, that the amount of cAMP and cGMP in the tissues may be influenced by the rapidity with which the animals were killed. Since the animals of all experimental groups were killed in the same way, we assume that the values in all experimental groups were influenced in the same way.

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Reprint Requests

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