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SHORT COMMUNICATION

Adenohypophyseal Ascorbic Acid: Influences of Oestradiol and Methylene Blue

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Summary

The ascorbic acid (AA) concentration in anterior pituitary and blood plasma was measured by the Roe-Kuether method in control rats and rats treated with oestradiol benzoate alone, methylene blue alone and with both oestradiol and methylene blue. We have found that methylene blue alone caused a significant drop in hypophyseal both AA and plasma AA concentrations. Methylene blue treatment prevented the increase in plasma AA concentration in oestradiol benzoate-treated rats.

Key words

Anterior pituitary – Ascorbic acid – Methylene blue – Oestradiol

The adenohypophysis occupies the second place as far as the tissue concentration of ascorbic acid (AA) is concerned: the first being the adrenals (for review see Kábrt 1982). While in the steroidogenic glands AA is suggested to play a role in oxido-reduction processes in the biosynthesis of steroid hormones, the function of AA in the anterior pituitary is completely unknown.

Our previous studies (Schreiber and Přibyl 1972) revealed that the content of AA in the anterior pituitary is decreased in oestrogen-induced anterior pituitary hypertrophy. We therefore performed a series of experiments in which oestrogens were combined with methylene blue.

Methylene blue is a thiazine dye used in medicine in the treatment of methaemoglobinaemia. Recently, the possibility of using methylene blue in the reperfusion syndrome (Salaris *et al.* 1991), ifosfamide encefalopathy (Kupfer *et al.* 1994) and endotoxaemic shock treatment (Paya *et al.* 1993) has appeared. Methylene blue can also play a role in the multiple transduction mechanism of dopamine (Enjalbert 1989), it partially blocks oestrogen-induced hypertrophy of anterior pituitary (Schreiber *et al.* 1993) and increases the blood thyroxine level (Nedvídková *et al.* 1994)

The ascorbic acid concentration decrease in the hypertrophic anterior pituitary after oestradiol benzoate treatment is well-known (Schreiber and Přibyl 1977). Methylene blue was able to modify the hypertrophic response of the anterior pituitary (Schreiber *et al.* 1993). We wondered whether there are also changes in ascorbic acid concentration during methylene blue treatment.

Four experiments were performed in Wistar male rats fed a standard laboratory diet. In all experiments, the rats were divided into four groups with 10 animals in each group: the controls, the group treated with oestradiol benzoate (Agofollin depot, 1 mg i.m., twice a week) alone, the group treated with methylene blue alone (0.5 % in the food) and the group simultaneously treated with methylene blue and oestradiol. The rats were killed by decapitation after three weeks of treatment. The anterior pituitaries were weighed and the adenohypophyseal and blood plasma ascorbic acid concentrations were measured by a modified method of Roe and Kuether (1943).

Table 1
The effect of chronic administration of methylene blue, oestradiol benzoate and oestradiol benzoate + methylene blue on adenohypophyseal and blood plasma ascorbic acid concentration

	Weight of adenohypophysis (mg/kg body weight)	Ascorbic acid in adenohypophysis (μg/mg adenohypophysis)	Ascorbic acid in blood plasma (mg/dl plasma)
Experiment No. 1			
1. Controls	27.6 ± 7.0 (2,4)	1.06 ± 0.34 (2,3,4)	
2. Oestradiol benzoate	107.6 ± 15.4 (1,3)	0.67 ± 0.14 (1)	
3. Methylene blue	40.1 ± 9.5 (2,4)	0.81 ± 0.11 (1)	
4. Oestradiol benzoate + Methylene blue	95.5 ± 23.1 (1,3)	0.64 ± 0.18 (1)	
Experiment No. 2			
1. Controls	49.5 ± 9.2 (2,4)	0.82 ± 0.16 (2)	2.07 ± 0.48 (2)
2. Oestradiol benzoate	106.1 ± 8.0 (1,3,4)	0.50 ± 0.09 (1,3,4)	5.23 ± 3.56 (1,3,4)
3. Methylene blue	57.1 ± 16.2 (2,4)	0.77 ± 0.09 (2)	1.12 ± 0.40 (2)
4. Oestradiol benzoate + Methylene blue	120.0 ± 15.9 (1,2,3)	0.87 ± 0.40 (2)	0.78 ± 0.09 (2)
Experiment No. 3			
1. Controls	43.1 ± 9.1 (2,4)	2.55 ± 0.48 (2,3,4)	2.50 ± 1.80 (3,4)
2. Oestradiol benzoate	98.8 ± 38.5 (1,3)	1.81 ± 0.57 (1,8)	3.50 ± 1.50 (3,4)
3. Methylene blue	25.0 ± 5.4 (2,4)	1.81 ± 0.39 (1,4)	1.25 ± 1.01 (1,2)
4. Oestradiol benzoate + Methylene blue	87.8 ± 43.1 (1,3)	1.13 ± 0.28 (1,2,3)	1.15 ± 0.90 (1,2)
Experiment No. 4			
1. Controls	60.8 ± 25.6 (2,4)	2.72 ± 0.48 (2,3,4)	2.56 ± 0.74 (3,4)
2. Oestradiol benzoate	160.4 ± 30.2 (1,3)	1.79 ± 0.64 (1)	3.00 ± 0.77 (3,4)
3. Methylene blue	64.1 ± 31.0 (2,4)	1.92 ± 0.42 (1)	1.70 ± 0.63 (1,2)
4. Oestradiol benzoate + Methylene blue	135.0 ± 60.7 (1,3)	1.81 ± 0.59 (1)	1.36 ± 0.52 (1,2)

Mean values ± 95 % confidence interval. The numbers in parentheses are the numbers of groups from which the relevant group is significantly different ($p < 0.01$)

Methylene blue (Table 1) alone caused a statistically significant drop in hypophyseal AA concentration. Rats treated with methylene blue alone had a significant drop in plasma AA concentration and methylene blue treatment prevented the increase in plasma AA concentration in oestradiol benzoate-

treated rats. The previous observations that the methylene blue partially blocks oestrogen-induced hypertrophy of anterior pituitary (Schreiber *et al.* 1993) were confirmed, although the significant suppression of the growth response appeared only in the absolute values (expressed in mg). When expressed in mg of adenohypophyseal weight per kg of body weight, no significant decrease was observed, probably because of the marked loss of body weight in rats simultaneously treated with oestradiol and methylene blue.

The mechanism of changes in the adenohypophyseal AA concentration is still unknown. As for the drop in blood plasma AA concentrations after methylene blue treatment, this may be due to an elevation of blood ceruloplasmin levels by methylene blue (Maruna *et al.* 1994). This hypothesis, as well as the reason why methylene blue prevented the increase in the blood plasma AA concentration induced by oestrogen treatment, require further investigations.

Between the second and third experiment we slightly altered the methodology, especially by using greater degree of the refrigeration of anterior pituitary homogenates. Indeed, the levels of AA in the samples clearly increased.

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Reprint Requests

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