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The Increase in cAMP and cGMP Levels in the Oestrogenized Rat Hypophysis

J. NEDVÍDKOVÁ, V. SCHREIBER

Institute of Endocrinology and Laboratory for Endocrinology and Metabolism, First Faculty of Medicine, Charles University, Prague

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Summary

Male and female rats were given oestradiol benzoate (1 mg s.c. twice a week for 3 weeks) and/or sodium nitroprusside (SN), a donor of nitric oxide (NO), which was administered in their food in amounts of 0.2 or 0.6 mg/rat/day. Neither oestradiol-induced hypertrophy of the hypophysis, nor the serum prolactin (PRL) level, was affected by the simultaneous administration of SN. The PRL content of the hypophysis rose after oestradiol in the males, but the increase was again uninfluenced by the simultaneous administration of SN and the cAMP content of the hypophysis – raised after oestradiol – was likewise unaffected. The amount of cGMP in the hypophysis after oestradiol rose only in males. Both the serum and the hypophyseal prolactin level were found to be correlated to the cAMP and the cGMP content of the hypophysis. It was found that the simultaneous administration of SN together with oestradiol slightly reduced the increase in the cGMP content of the hypophysis elicited with oestradiol treatment only.

Key words

Oestradiol – Hypophysis – Sodium nitroprusside – cAMP – cGMP – Prolactin

Introduction

In rats, the administration of oestradiol is followed by marked hypertrophy of the adenohypophysis, which can be inhibited with the thyroid hormones, anti-oestrogens, testosterone and dopaminergic agonists (lisuride) and is stimulated by dopaminergic antagonists (e.g. perphenazine) (for review see Pacák *et al.* 1991). The dopaminergic agonist lisuride also inhibits oestrogen-induced changes in the histological structure of the adenohypophysis (Dušková and Schreiber 1989). The main functional change in the oestrogenized hypophysis is an increase in prolactin (PRL) secretion. A series of reports on the relationship between PRL secretion and the cAMP content of the adenohypophysis (Gautvik *et al.* 1980, Swenen and Denef 1982, Schettini *et al.* 1985, Kolp *et al.* 1991, Judd and MacLeod 1991), or between the cAMP content of the hypophysis and PRL and somatotropin secretion (Swenen *et al.* 1985, Szabo *et al.* 1990), are available.

We therefore decided to determine cAMP and cGMP levels in the rat hypophysis after the administration of oestrogens. Since cGMP is the "third messenger" in the sequence acetylcholine (first messenger) – NO synthase – NO (second messenger) – guanylate cyclase – cGMP (third messenger), we combined the administration of oestradiol with the administration of sodium nitroprusside, a donor of nitric oxide (NO). NO plays a role in neuroendocrine reactions (Bredt *et al.* 1990), as well as in vascular reactions (Katsuki *et al.* 1977). It is well known that the half-life of intravenously administered sodium-nitroprusside is extremely low but we wanted to examine, whether the peroral administration with sustained blood levels would produce any effect.

Material and methods

Male and female rats (Wistar offspring) with an initial body weight of 158–193 g (see the tables) were injected subcutaneously twice a week with oestradiol benzoate (10 mg/kg) in the form of an aqueous microcrystal suspension (Agofolin Depot, SPOFA) and/or were given – in their food – sodium nitroprusside in doses of 0.2 mg/rat/day (first experiment) or 0.6 mg/rat/day (second experiment). The experiments lasted three weeks.

At the end of the experiments, the rats were killed by decapitation: the adenohypophyses were quickly removed, weighed on a torsion balance and frozen in liquid N_2. The blood was collected and the serum, separated by centrifugation, was stored frozen at -20 °C until required for prolactin assay (RIA with rat PRL NIADDK rPRL-I-5 for radioiodination, NIADDK-rPRL-RP-3 as the reference preparation and rPRL NIADDK-anti-rPRL-S-8 antiserum: see acknowledgements) in the first experiment and for thyroxine assay (Nedvídková and Felt 1977) in the second.

For cAMP and cGMP estimation, the frozen adenohypophyses were homogenized in a Braun Potter-Elvehjem all-glass homogenizer (10 strokes, 1 000 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 at 2 000 x *g* and 4 °C in a type GPR Beckman centrifuge. The supernatant was collected and frozen at -20 °C until required for cAMP and cGMP assay. cAMP and cGMP levels were estimated by means of radioimmunoassay kits (both supplied by the Institute for the Production, Development and Utilization of Radioisotopes, Prague).

The means of all the values and the 95 % confidence intervals were computed and the significance of differences of the means was evaluated by an analysis of variance, using Duncan's test (Duncan 1955). In experiment No. 1, the correlation coefficients and their significance were also calculated.

Results

The results are shown in Tab. 1-3. Oestradiol distinctly increased the weight of the adenohypophysis – a reaction which was influenced only nonsignificantly or not at all by sodium nitroprusside (SN). The serum prolactin (PRL) level, which rose after oestradiol, was unaffected by SN. After oestradiol, the PRL content of the hypophysis rose only in males and the increase was not affected by SN.

The cAMP content of the adenohypophysis again rose only in males, but the difference per pmol/mg was significant in the first experiment only. SN did not influence oestradiol-induced changes in either the first or the second experiment. The amount of cGMP in the adenohypophysis was also inhibited by the simultaneous administration of SN.

In the first experiment, significant correlations between the serum prolactin level and the cAMP and cGMP content of the adenohypophysis, and between the prolactin content of the hypophysis and its cAMP and cGMP content, were found.

As regards thyroxine levels in the second experiment, some changes did occur (a decrease after oestradiol and sodium nitroprusside compared with the controls), but there was no difference between group 2 (oestradiol) and group 4 (oestradiol + sodium nitroprusside).

Discussion

When adenohypophyseal cells are cultivated *in vitro*, a correlation between stimulation of the cAMP (and of the Ca^{2+} and calmoduline) content and prolactin secretion can be observed (Schreiber *et al.* 1985). Dopamine, which inhibits prolactin secretion, likewise inhibits the *in vitro* production of cAMP in adenohypophyseal cell cultures (Swenen and Denef 1982); in this study, dopamine antagonists (spiperone, domperidone) also inhibited the effect of dopamine on the cAMP content of adenohypophyseal cells *in vitro*.

The cAMP content of the adenohypophysis is further related to proteosynthesis in the gland (Labrie *et al.* 1973); in addition to cAMP, cIMP and cGMP also stimulate ribosome-associated protein kinase activity, although cAMP is the most effective (to achieve the same effect, a tenfold higher cIMP concentration and a 500-fold higher cGMP concentration was needed).

It is clear from our results that the post-oestradiol increase in adenohypophyseal weight in males (251 % in the first experiment and 330 % in the second) is much greater than in females (160 % in the first experiment). The increase in the cAMP content was correspondingly greater – both absolutely (pmol/hypophysis) and relatively (pmol/mg hypophysis) in the first experiment, though only absolutely in the second. In females, the cAMP content of the hypophysis – like the cGMP content – did not increase after oestradiol, whereas in males the cGMP content always rose, both absolutely and relatively. Likewise the ratio of cAMP/cGMP was significantly decreased only in males, not in females. It is thus possible that sex-linked differences in cGMP reactivity in the hypophysis exist. At all events, the simultaneous administration of sodium nitroprusside together with oestradiol slightly inhibits the reaction of the increase in the cGMP content of the hypophysis.

Table 1

Results of Exp. No.1. Means ± 95% confidence intervals. The figures in brackets denote groups with statistically different means (Duncan test)

Group	1 Controls (n=6)	2 Oestradiol (n=6)	3 Sodium nitroprusside (n=7)	4 Oestradiol + sodium nitroprusside (n=7)
Body weight, g, init.	187 ± 6.43	185 ± 3.95	188 ± 2.26	187 ± 5.25
final	324 ± 26.10(2,4)	227 ± 33.12(1,3)	314 ± 20.39(2,4)	225 ± 16.88(1,3)
Adenohypophysis, mg	13.37 ± 1.28(2,4)	23.48 ± 8.54(2,4)	14.12 ± 1.86(2,4)	21.89 ± 5.30(1,3)
mg/kg	41.62 ± 4.91(2,4)	103.32 ± 23.18(1,3)	45.46 ± 5.67(2,4)	90.26 ± 25.75(1,3)
Prolactin, s., ng/ml	9.82 ± 5.40(2,4)	54.38 ± 31.80(1,3)	8.59 ± 6.43(2.4)	99.51 ± 61.25(1,3)
Prolactin, adenohypophysis, ng	5.00 ± 3.27(2,4)	25.42 ± 14.06(2,4)	7.70 ± 5.90(2,4)	26.04 ± 17.15(1,3)
ng/mg	0.36 ± 0.22(2)	1.07 ± 0.41(1,3)	0.57 ± 0.40(2)	1.27 ± 0.93(1,3)
cAMP, pmol/adenohypophysis	48.93 ± 12.42(2,4)	120.54 ± 35.61(1,3)	42.84 ± 7.99(2,4)	119.65 ± 29.70(1,3)
pmol/mg	3.63 ± 0.79(2,4)	5.18 ± 0.97(1,3)	2.89 ± 0.49(2,4)	5.33 ± 0.96(1,3)
cGMP, pmol/adenohypophysis	4.82 ± 1.19(4)	26.50 ± 24.72(1,3)	4.73 ± 0.62(4)	15.98 ± 7.50(1,3)
pmol/mg	0.36 ± 0.06(2,4)	1.14 ± 0.31(1,3,4)	0.34 ± 0.07(2,4)	0.71 ± 0.15(1,3)

Correlation:
Prolactin serum : cAMP pmol/mg adenohypophysis r = 0.6571 ($p<0.01$)
Prolactin serum : cGMP pmol/mg adenohypophysis r = 0.5693 ($p<0.01$]
Prolactin ng/mg hypophysis : cAMP pmol/mg hypophysis r = 0.6623 ($p<0.01$)
Prolactin ng/mg hypophysis : cGMP pmol/mg hypophysis r = 0.6326 ($p<0.01$)

It is known from the literature (e. g. Kutsuki *et al.* 1977) that NO generated from the nitrates increases the cGMP content in tissues. As mentioned in the introduction, the possibility that sodium nitroprusside could act as a donor of NO groups in an *in vivo* experiment seems improbable, owing to short half life of the substance. Transformation of sodium nitroprusside should be taken into account. Manzoni *et al.* (1992) showed that sodium nitroprusside can also act through ferrocyanide ions, the other side-product of sodium nitroprusside destruction and that this product can have strong actions independent of NO. Therefore, care should be taken when using sodium nitroprusside as a typical NO substitute. The cyanate metabolites of sodium nitroprusside are goitrogenous, however, but we did not find any significant difference in thyroxine levels in oestradiol- and oestradiol + sodium nitroprusside treated animals. The serum level of thyroxine was, however, significantly lower in sodium nitroprusside treated rats than in controls. The difference between male and female rats in the reaction of cAMP + cGMP hypophyseal levels merits further attention. It should be mentioned that the other experiment with females provided negative results, which are not mentioned here.

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Table 2
Results of Exp. No.1. Female rats. Means ± 95% confidence intervals. The figures in brackets denote groups with statistically different means (Duncan's test)

Group	1 Controls (n = 6)	2 Oestradiol (n = 6)	3 Sodium nitroprusside (n = 7)	4 Oestradiol + sodium nitroprusside (n = 7)
Body weight, g, init.	158 ± 5.42	160 ± 6.97	167 ± 7.29	162 ± 7.49
final	253 ± 39.85(2,4)	187 ± 15.99(1,3)	250 ± 17.51(2,4)	197 ± 27.87(1,3)
Adenophysis, mg	14.77 ± 2.15	17.44 ± 3.60	16.11 ± 3.11	18.06 ± 3.32
mg/kg	58.62 ± 7.26(2,4)	93.53 ± 19.33(1,3)	64.24 ± 10.0(2,4)	91.40 ± 14.34(1,3)
Prolactin, s., ng/ml	12.02 ± 9.77(4)	27.20 ± 21.05	10.33 ± 9.18(4)	37.91 ± 18.20(1,3)
Prolactin, adenohypophysis, ng	11.88 ± 11.57	17.78 ± 16.13	15.27 ± 7.42	24.26 ± 15.59
ng/mg	0.90 ± 1.21	1.04 ± 1.5	0.97 ± 0.46	1.15 ± 0.59
cAMP, pmol/adenohypophysis	72.25 ± 20.04	72.10 ± 10.65	68.54 ± 21.22	83.15 ± 27.14
pmol/mg	4.88 ± 1.06	4.18 ± 0.59	4.19 ± 0.58	4.67 ± 1.0
cGMP, pmol/adenohypophysis	7.10 ± 1.83	8.83 ± 3.97	7.30 ± 1.09	10.13 ± 3.63
pmol/mg	0.4 ± 0.14	0.50 ± 0.20	0.51 ± 0.09	0.56 ± 0.15

Table 3

Results of Exp. No. 2. Male rats. Means ± 95% confidence intervals. The figures in brackets denote groups with statistically different means (Duncan's test)

Group	1 Controls (n=6)	2 Oestradiol (n=6)	3 Sodium nitroprusside (n=7)	4 Oestradiol + sodium nitroprusside (n=7)
Body weight, g, init.	193 ± 3.49	193 ± 2.87	192 ± 2.87	191 ± 2.11
final	274 ± 7.60(2,4)	182 ± 8.86(1,3)	291 ± 12.46(2,4)	180 ± 4.15(1,3)
Adenophysis, mg	8.88 ± 0.81(2,4)	19.63 ± 2.08(1,3)	9.84 ± 1.12(2,4)	18.18 ± 1.46(1,3)
mg/kg	32.2 ± 2.93(2,4)	109.4 ± 10.17(1,3)	33.84 ± 3.72(2,4)	102.5 ± 9.39(1,3)
cAMP, pmol/adeno-hypophysis	34.68 ± 6.05(2,4)	92.01 ± 21.41(1,3)	36.43 ± 8.13(2,4)	76.26 ± 19.12(1,3)
pmol/mg	3.86 ± 0.44	4.52 ± 0.84	3.70 ± 0.39	4.00 ± 0.59
cGMP, pmol/adeno-hypophysis	8.53 ± 1.15(2,4)	35.49 ± 7.59(1,3,4)	8.18 ± 0.65(2,4)	25.44 ± 5.36(1,3)
pmol/mg	0.96 ± 0.11(2,4)	1.67 ± 0.31(1,3,4)	0.86 ± 0.09(2,4)	1.32 ± 0.26(1,3)
Thyroxine, s, nmol/l	65.95 ± 7.78(2,4)	47.48 ± 5.34(1)	51.51 ± 4.64(1,4)	42.57 ± 6.84(1,3)

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Reprint Requests
Dr. J. Nedvídková, Institute of Endocrinology, CS-116 94 Prague 1, Národní 8.