Further Investigations on the Trophic Effect of 3′:5′-Cyclic Adenosine Monophosphate and 3′:5′-Cyclic Guanosine Monophosphate on the Zona Glomerulosa of Hypophysectomized Rat Adrenal Cortex

In earlier contribution[1] it was reported that ACTH and 3′:5′-cyclic adenosine monophosphate (cAMP) enhance the growth of the rat adrenal zona glomerulosa. Since ACTH was found to stimulate adenyl cyclase in capsular adrenal (zona glomerulosa) in vitro[2] we suggested that cAMP can function as an intracellular mediator of the glomerulotrophic action of ACTH. More recently we have shown that also 3′:5′-cyclic guanosine monophosphate (cGMP) exerts trophic action on the rat zona glomerulosa[3]. Since phosphodiesterase (the enzyme destroying the biologic activity of cAMP) is active against all the purine cyclic nucleotides[4], the possibility that the cGMP glomerulotrophic action consist in the inhibition of this enzyme remained, however, to be settled. Furthermore, according to what reported for the rat zona fasciculata[5–8], neither cAMP nor CGMP were found to parallel completely the trophic effect of ACTH on glomerulosal cells.

It therefore seemed worthwhile to investigate, by morphometric methods and electron microscopy, both the effects of theophylline, a phosphodiesterase inhibitor[4], and of the simultaneous administration of cAMP and cGMP on the maintenance of the growth of hypophysectomized rat zona glomerulosa.

Materials and methods. The animals used in this investigation were young adult male albino rats (Sprague-Dawley) weighing about 120–140 g. The rats were maintained on Purina rat-mouse chow and tap water ad libitum. The animals were divided into 7 experimental groups. 5 groups were hypophysectomized by the parapharyngeal approach. The completeness of the operation was checked at autopsy.

On the 6th postoperative day, 4 hypophysectomized groups were given ip injections of 20 mg cAMP/kg, 20 mg cGMP/kg, 10 mg cAMP plus 10 mg cGMP/kg, or 60 mg theophylline/kg (Sigma Chemical Company, St. Louis, Mo.) for 5 consecutive days. An intact group received ip injections of 60 mg theophylline/kg for 5 consecutive days. The other 2 groups (intact and hypophysectomized) received daily ip injections of normal saline.

Sliced pieces of the right adrenal were processed for electron microscopy. Thick and thin sections were cut at the level of the zona glomerulosa and observed with the light microscope and the Hitachi HU-12 electron microscope, respectively.

The percent of cellular volume occupied by the various organelles and the 'concentration' of smooth (SER) and rough (RER) endoplasmic reticulum membranes and

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Changes in morphometric parameters of rat *zona glomerulosa* after hypophysectomy, and treatment with theophylline and 3′:5′-cyclic nucleotides

Parameters		Control (6)	Hypophysectomy (4)	Theophylline (6)	Hypohysectomy-theophylline (5)	Hypophysectomy-cAMP (5)	Hypophysectomy-cGMP (6)	Hypophysectomy-cAMP + cGMP (5)
Volume of cells	μm³	624.5 ± 70.6	376.5 ± 39.7	736.9 ± 78.4	384.7 ± 40.1	543.4 ± 54.6	532.2 ± 53.2	648.7 ± 74.5
	p_1		<0.01	<0.01	<0.01	<0.05	<0.01	N.S.
	p_2				N.S.	<0.01	<0.01	<0.01
Volume of nuclei	μm³	105.2 ± 11.5	81.9 ± 7.8	136.9 ± 13.2	83.2 ± 9.1	98.1 ± 10.2	96.3 ± 9.7	109.4 ± 11.9
	p_1		<0.01	<0.01	<0.01	N.S.	N.S.	N.S.
	p_2				N.S.	<0.02	<0.02	<0.01
Volume of mitochondrial fraction	μm³	138.1 ± 14.1	86.8 ± 9.4	156.6 ± 16.2	88.3 ± 10.3	109.7 ± 11.2	106.7 ± 10.6	142.3 ± 14.6
	p_1		<0.01	<0.05	<0.01	<0.01	<0.01	N.S.
	p_2				N.S.	<0.01	<0.01	<0.01
Surface of mitochondrial cristae	μm²	447.4 ± 50.6	268.7 ± 28.1	507.7 ± 54.3	265.3 ± 28.6	379.8 ± 40.7	358.7 ± 40.4	448.9 ± 51.7
	p_1		<0.01	<0.05	<0.01	<0.02	<0.01	N.S.
	p_2				N.S.	<0.01	<0.01	<0.01
Surface of SER	μm²	2209.2 ± 209.4	1030.2 ± 111.2	2841.8 ± 293.7	1079.6 ± 110.6	1983.3 ± 198.1	1961.5 ± 197.4	2230.2 ± 211.8
	p_1		<0.01	<0.01	<0.01	<0.05	<0.05	N.S.
	p_2				N.S.	<0.01	<0.01	<0.01
Surface of RER	μm²	109.6 ± 15.7	106.8 ± 14.8	111.4 ± 17.2	108.9 ± 17.3	109.7 ± 14.8	105.9 ± 17.5	107.4 ± 16.5
	p_1		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	p_2				N.S.	N.S.	N.S.	N.S.
Volume of lipid compartment	μm³	41.4 ± 5.6	62.7 ± 7.0	43.7 ± 4.8	60.8 ± 6.9	45.4 ± 5.1	46.3 ± 5.6	40.4 ± 5.3
	p_1		<0.01	N.S.	<0.01	N.S.	N.S.	N.S.
	p_2				N.S.	<0.01	<0.01	<0.01

Animals were treated as described in the text. The number of rats in each group is indicated in parentheses. Each value represents the group mean ± S.E. p_1, level of significance of the difference from the control group. p_2, level of significance of the difference between hypophysectomized and hypophysectomized theophylline-, and cyclic nucleotide-treated groups. N.S., not significant ($p > 0.05$).

mitochondrial cristae (i.e., μm^2 of SER, RER, and mitochondrial cristae/μm^3 of cytoplasm) were estimated by conventional stereologic methods[9], using the sampling procedures previously reported[1]. The absolute amount of the various organelles in the individual glomerulosal cell was obtained by determining the average cellular volume with the same indirect approach detailed in an earlier contribution[10]. Student's *t*-test was used for the statistical evaluation of results.

Results and discussion. According to what was reported previously[1,2], both cAMP and cGMP were found to reverse, although not completely, the hypophysectomy-induced atrophy of the zona glomerulosa: in fact, the volume of cells, nuclei, mitochondrial compartment as well as the surface of SER and mitochondrial cristae display a significant increase, while the volume of lipid compartment shows a considerable decrease (Table).

The possibility that the trophic action of cGMP consists in the competitive inhibition of adrenal phosphodiesterase can be disregarded, since theophylline, while significantly enhancing the growth of intact rat zona glomerulosa (presumably by inhibiting the cAMP biologic degradation), does not exert trophic action on 11-days hypophysectomized rat glomerulosal cells, in which the ATP-adenyl cyclase system is no longer functioning[11] (Table).

The Table shows that the simultaneous chronic administration of emidoses of cAMP and cGMP induces full maintenance of the growth of hypophysectomized rat zona glomerulosa. This finding could be reasonably explained by assuming that cGMP potentiates the cAMP-action by inhibiting adrenocortical phosphodiesterase. However, the following pieces of evidence are against this possibility: 1. the doses of single cyclic nucleotide administered in the present study were very high, and 2. cGMP was not found to act sinergistically at high doses of cAMP[12].

On this basis, our data seem to be consistent with the hypothesis that cGMP, like cAMP, can act as an intracellular mediator of the trophic action of ACTH on the rat zona glomerulosa.

However, it is to be stressed that, since rat adrenal guanyl cyclase is not sensitive to ACTH[13], the challenge hypothesis that cGMP can function as an intracellular mediator of other glomerulotrophic factors (e.g., renin-angiotensin system) cannot be excluded, at present. Studies are in progress to settle this point[14].

Riassunto. Oltre il cAMP, anche il cGMP esercita azione trofica sulla zona glomerulare di ratto. Entrambi i nucleotidi ciclici però, anche se somministrati in alte dosi, non annullano completamente gli effetti dell'ipofisectomia. La somministrazione simultanea di cAMP a cGMP provoca, invece, pieno mantenimento del trofismo della zona glomerulare di ratto ipofisectomizzato. Viene discussa la possibilità che entrambi i nucleotidi ciclici intervengano nella mediazione intracellulare dell'azione glomerulotrofica dell'ACTH.

G. MAZZOCCHI, G. G. NUSSDORFER, P. REBUFFAT, A. S. BELLONI and V. MENEGHELLI

Istituto di Anatomia Umana Normale
Via A. Gabelli, 37, I-35100 Padova (Italy), 25 July 1973.

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Estrogen-Induced Phosvitin Synthesis in Cultured Chick Embryo Liver Cells

Phosvitin is a yolk protein of certain oviparous vertebrates. In birds, it is synthesized in liver of laying hens and transported in the blood to the developing oocyte[1,2]. In male and immature birds, hepatic synthesis of phosvitin can be induced by estrogen administration[3,4]. Such a system has been extensively used for studying the nature of the mechanism involved in steroid hormones regulation of specific proteins synthesis in vivo[2,5-9]. An in vitro system could improve the experimental opportunities for these investigations. We have therefore investigated the possibility of stimulating phosvitin synthesis in chick embryo liver cultures.

Materials and methods. Livers removed from 14-day old chick embryos were gently cut into small pieces, washed in Tyrode's solution and dissociated in 0.25% buffered trypsin (Difco) at room temperature for 25–30 min. The cells were then filtered through a nylon mesh, centrifuged (35 *g*, 10 min) and resuspended in 199 (Gibco, Grand Island, N.Y.) with 20% added calf serum and penicillin 200 units/ml. 5 ml of cell suspension (4×10^6 cells/ml) were put in flasks and incubated at 37 °C. Cells obtained from the same chick embryos' hearts were used as control.

Estradiol-17-β (Merck; in propylene glycol, 50 $\gamma/\mu l$) was incorporated in the medium at the final concentration of 500 γ/culture. The time schedule was recorded in Figure 1a. 45 liver cultures (25 test and 20 control ones) and 20 heart cultures (10 test and 10 control ones) were carried out and were observed daily with an inverted Reichert microscope. Slides were fixed in methanolic alcohol and stained with May-Grümwald-Giemsa.

After collection and centrifugation (35 g-10 min) the culture medium was dialized extensively against 0.9% NaCl, lyophilized, dissolve in 0.5 ml 0.9% NaCl and then examined by means of the Ouchterlony[10] double gel immunodiffusion and the immunoelectrophoretic micro-

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