DIFFERENT PLASMA MEMBRANE EFFECTS OF ADRENOCORTICOTROPIC HORMONE AND CYCLIC NUCLEOTIDES IN NORMAL AND MALIGNANT ADRENAL CELLS

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1. Introduction

Correlative studies of the biochemical control mechanisms in the normal adrenal [1,2] and adrenocortical carcinoma cells [3], have revealed various biochemical lesions both before and after the events leading to the cleavage of the cholesterol side chain. These metabolic lesions are responsible for the altered, but unique, ACTH-controlled system in these cells. These abnormalities have been observed at the level of plasma membrane [4], adenylate cyclase system [5,6], phosphodiesterase activity [7], and in the steroidogenic biosynthetic transformations of (20 S)-20-hydroxycholesterol [8,9], pregnenolone, progesterone and deoxycorticosterone [10] to corticosterone. During the course of these investigations it was observed [4] that, in an isolated adrenal cell and adrenocortical carcinoma cell, ACTH, in a unique way, stimulates the transport of deoxycorticosterone across the plasma membrane. That the phenomenon of stimulation by the polypeptide hormone was different from that of N^6-$O^{2'}$-dibutyryl cyclic AMP was evidenced by the fact that although this cyclic nucleotide stimulated the membrane phenomenon in the normal cell, it failed to do so in the malignant cell [4]. This clearly established that the modes of action of ACTH and cyclic AMP on the plasma membrane were different and independent of each other.

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2. Materials and methods

Isolated adrenal and adrenocortical carcinoma cell preparations were made as previously described [1–3]. The method of incubation with double labeled radioactive precursors was as previously described [10]. Incubation was carried out in 3 Teflon flasks. Each flask contained 20 ml isolated adrenal tumor cell suspension [3] prepared from 1.5 g adrenal tumor tissue or the same amount of cell suspension obtained from 16 adrenal glands of the rat [1,2]. In addition, to the appropriate cell suspension, the first flask contained a mixture of 25 μCi of [1,2-$^3H_2$]deoxycorticosterone (specific activity 46.8 Ci/mmol) and 1 μCi of [4-^{14}C]deoxycorticosterone (specific activity 59.8 mCi/mmol) (^{3}H/^{14}C ratio 25.0); the second flask contained 25 μCi of [^{3}H]deoxycorticosterone and cyclic AMP, cyclic GMP or cyclic IMP, and the third flask 1 μCi of [4-^{14}C]deoxycorticosterone. The incubation was continued for 150 min and the reaction was stopped by the addition of 75 ml of methylene chloride into each flask. The contents of the second flask were mixed with contents of the third flask. To the reaction mixture of each flask, 10 mg of corticosterone and 15 mg of deoxycorticosterone were added and the products processed identically.

The experiment with the preloading of the cells with [^{3}H]deoxycorticosterone was conducted as previously described [11]. Twenty millilitres of suspended cells obtained from adrenal glands of 32 rats were preincubated for 30 min with 50 μCi of [^{3}H]deoxycorticosterone. The cell pellet obtained by centrifugation was washed with 20 ml Krebs–Ringer–bicarbonate

Table 1
Effect of cAMP on the incorporation of [^{3}H]deoxycorticosterone into corticosterone in normal isolated adrenal cells

Crystallization	^{3}H/^{14}C ratio of corticosterone in		% Stimulation
	Flask 1	Flask 2 + 3	
1st	25.33	36.47	
2nd	25.61	37.13	
3rd	26.23	37.50	44

The ^{3}H/^{14}C ratios of the products obtained after the incubation of [1,2-$^3H_2$, 4-^{14}C]-deoxycorticosterone with isolated adrenal cells. Conditions of the experiment were similar to the experiment in table 2.

buffer which contained albumin and glucose, and the cells were resuspended in 60 ml of Krebs–Ringer–bicarbonate buffer containing albumin and glucose. The cell suspension was divided into three flasks. The control flask contained 20 ml of suspended cells and [^{3}H]deoxycorticosterone, and the other two flasks contained cyclic GMP or cyclic IMP. The incubation was carried out for 150 min and the reaction mixture processed as mentioned earlier.

Deoxycorticosterone and corticosterone were purified by thin-layer chromatography. The isolated deoxycorticosterone was acetylated [10], further purified by thin-layer chromatography [9,10], and crystallized from acetone–*n*–hexane to constant specific activity. The purified corticosterone was crystallized from acetone–ligroin until the specific activity and (^{3}H/^{14}C) ratio were constant.

3. Results and discussion

The present investigation shows that cyclic AMP, cyclic GMP and cyclic IMP, in contrast to the tumor cell, stimulate the incorporation of deoxycorticosterone into corticosterone in isolated adrenal cell (table 1–3).

Table 2
Effect of cGMP on the incorporation of [^{3}H]deoxycorticosterone into corticosterone in normal isolated adrenal and adrenocortical carcinoma cells

Isolated cells from	Crystallization	^{3}H/^{14}C ratio of corticosterone from		% Stimulation
		Flask 1	Flask 2 + 3	
Normal adrenal	1st	25.18	43.05	
	2nd	25.63	42.33	
	3rd	25.97	43.70	68
Adrenocortical carcinoma	1st	25.03	26.13	
	2nd	25.88	27.17	
	3rd	26.70	27.76	0

The ^{3}H/^{14}C ratios of corticosterone obtained after the incubation of [1,2-$^3H_2$, 4-^{14}C], deoxycorticosterone with isolated adrenal and adrenocortical carcinoma cells. Incubation was carried out in 3 flasks containing 20 ml isolated adrenal or adrenocortical carcinoma cell preparation as mentioned in Materials and methods. Flask 1 contained a mixture of [1,2-$^3H_2$]deoxycorticosterone (25 μCi) + (4-^{14}C]deoxycorticosterone (1 μCi) (^{3}H/^{14}C ratio 25.00; flask 2 contained [1,2-$^3H_2$]deoxycorticosterone (25 μCi) + cGMP (10 mM) and flask 3 contained [4-^{14}C] deoxycorticosterone (1 μCi). The incubation was 2.5 h and the reaction was stopped by the addition of 75 ml methylene chloride to each flask. The contents of the second and third flask were mixed and corticosterone isolated as described in Materials and methods.

Table 3
Effect of cIMP on the incorporation of [^{3}H]deoxycorticosterone into corticosterone in normal isolated adrenal and adrenocortical carcinoma cells

Isolated cells from	Crystallization	^{3}H/^{14}C ratio of corticosterone in		% Stimulation
		Flask 1	Flask 2 + 3	
Normal adrenal	1st	25.18	88.13	
	2nd	25.63	89.28	
	3rd	25.97	90.89	250
Adrenocortical carcinoma	1st	25.03	25.44	
	2nd	25.88	25.12	
	3rd	26.70	25.63	0

The ^{3}H/^{14}C ratios of the products obtained after the incubation of [1,2-$^3H_2$, 4-^{14}C]deoxycorticosterone with isolated adrenal and adrenocortical carcinoma cells. Conditions of the experiment were similar to the experiment in table 2.

The evidence has been provided that this requires the interposition of the plasma membrane, since preloading the cells eliminates cyclic nucleotides stimulation. To eliminate the possibility that the nonstimulatory effect of cyclic nucleotides in the preloaded cells might be due to the insufficient availability of the substrate, deoxycorticosterone, the latter steroid was isolated, purified and crystallized as the 21-acetyl derivative. The results in table 4 show there was still about 90% of the unused substrate, deoxycorticosterone, in the incubation system.

In conclusion, the results of the present investigation, together with the previous study [4], indicate ACTH, cyclic AMP, cyclic GMP and cyclic IMP, the

Table 4
Effect of cGMP and cIMP on the incorporation of [^{3}H]deoxycorticosterone into corticosterone in preloaded normal isolated adrenal cells

Compound	Crystallization	^{3}H dpm of compound from		
		Control	+cGMP	+cIMP
Deoxycorticosterone acetate	1st	182 192	168 968	178 330
Corticosterone	1st	21 820	18 640	20 100
	2 nd	20 130	17 090	19 250
	3rd	18 040	16 600	18 210

The total ^{3}H-dpm of the products (and their derivatives) obtained after the incubation of [1,2-$^3H_2$]deoxycorticosterone with isolated adrenal cells. Twenty milliliters of isolated adrenal cell preparation obtained from adrenals of 32 rats was preincubated with 50 μCi of [1,2-$^3H_2$]deoxycorticosterone for 30 min. The cells were washed and resuspended in 60 ml of Krebs–Ringer–bicarbonate buffer containing albumin and glucose. Flask 1, used as a control, contained 20 ml aliquot of cell suspension, flask 2 contained 20 ml aliquot of cell suspension + cGMP (10 mM), whereas to flask 3, in addition to cell suspension, (20 ml) was added cIMP (10 mM). The incubation was for 2.5 h and the reaction was stopped by the addition of 75 ml methylene chloride to each flask. Deoxycorticosterone and corticosterone were isolated and purified by thin-layer chromatography and the crystallized to constant specific activity as described earlier.

agents which stimulate steroidogenesis [1] in an isolated adrenal cell also stimulate the transport phenomenon of deoxycorticosterone across the plasma membrane. In the tumor, however, ACTH stimulates the membrane effect [4] but the cyclic nucleotides are incapable of doing so. Previously, it has been shown [3] that neither ACTH nor cyclic AMP, cyclic GMP or cyclic IMP, are able to stimulate corticosterone synthesis in the tumor cells. Recently [12,13] the protein kinase enzyme, which binds cyclic AMP but is unable to activate the cyclic AMP-dependent phosphorylation process, has been isolated and purified from the adrenocortical carcinoma. It has been proposed that the lack of cyclic AMP-stimulated steroidogenesis is due to the defective protein kinase enzyme [12,13]. It is therefore quite probable that the inability of the cyclic nucleotides to influence membrane transport process in the malignant cell is due to the lack of the cyclic nucleotide-dependent phosphorylation phenomenon.

These studies, therefore, indicate that not only the two types of membrane transport processes are different in the malignant and normal cell, but they also indicate that the phenomenon of ACTH-activated transport system is quite distinct from the cyclic nucleotide-stimulated system in a normal cell. Since in the tumor, where there is a defective protein kinase system, ACTH does stimulate but the cyclic nucleotides do not stimulate the membrane-dependent transport system, this indicates that the ACTH-stimulated membrane phenomenon does not involve the obligatory protein kinase system but for the cyclic nucleotide-activated system this is a preprequisite. These factors have been depicted in fig.1.

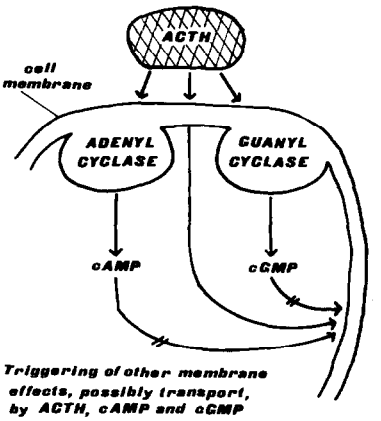

Fig.1.

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References

[1] Kitabchi, A. E. and Sharma, R. K. (1971) Endocrinology 88, 1109–1116.
[2] Sharma, R. K., Hashimoto, K. and Kitabchi, A. E. (1972) Endocrinology 91, 994–1003.
[3] Sharma, R. K. and Hashimoto, K. (1972) Cancer Res. 32, 666–674.
[4] Sharma, R. K. (1974) FEBS Lett. 38, 197–201.
[5] Brush, J. S., Sutliff, L. S. and Sharma, R. K. (1974) Cancer Res. 34, 1495–1502.
[6] Schorr, I. and Ney, R. L. (1971) J. Clin. Invest. 50, 1295–1300.
[7] Sharma, R. K. (1972) Cancer Res. 32, 1734–1736.
[8] Sharma, R. K. and Brush, J. S. (1973) Arch. Biochem. Biophys. 156, 560–562.
[9] Sharma, R. K. and Brush, J. S. (1974) Biochem. Biophys Res. Commun. 56, 256–263.
[10] Sharma, R. K. (1973) Eur. J. Biochem. 32, 506–512.
[11] Sharma, R. K. (1973) J. Biol. Chem. 248, 5473–5476.
[12] Sharma, R. K. and Ahmed, N. K., Tenth International Congress of Biochemistry, Hamburg, Germany July, 1976.
[13] Sharma, R. K. (1976) in: 'Control Mechanisms of Cancer' (Criss, W. E., Ono, T. and Sabine, J. R., eds) pp. 109–124, Raven Press, New York.