RAT LIVER ADENYLATE CYCLASE AND PHOSPHODIESTERASE DEPENDENCE ON Ca^{2+} AND ON CYTOPLASMIC FACTORS DURING LIVER REGENERATION

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1. Introduction

The activity of the enzymes known to control the intracellular level of cyclic AMP – adenylate cyclase and phosphodiesterase – is modulated by several factors acting both on the inner and on the outer surface of the membrane. Rodbell [1] showed that adenylate cyclase is activated by GTP and by its analogue GMP–PNP; Ho et al. [2] showed that a cytoplasmic factor is an antagonist of the lipolytic effect of adrenaline because it acts as a feedback inhibitor of adenylate cyclase; Gill and King [3] observed that a cytoplasmic factor may be involved in the activation of adenylate cyclase by cholera toxin. Sanders et al. [4] reported that the hormone response of adenylate cyclase is enhanced by the soluble fraction in the cardiac muscle. Pecker and Hanoune [5] and Doberska and Martin [6] observed the same behaviour in rat liver plasma membranes. Furthermore a non dialysable and heat-stable protein has been detected both in the soluble fraction [7,8] and in some particulate fractions and its action has been shown to be Ca^{2+}-dependent. This protein is known to be present, as modulator of high K_m phosphodiesterase activity, in different mammalian [10] and avian [11] tissues and in fibroblasts and it has been pointed out that correlations between phosphodiesterase and activator may change in relation to the different conditions, i.e. during development [10,11]. Otherwise the adenylate cyclase modulation exerted by this protein has been observed in nervous tissue [13,14,15], and in glial tumor cells [16].

The aim of the present research work was to investigate the possible phosphodiesterase and adenylate cyclase control exerted by Ca^{2+} and by rat liver soluble fraction both in normal conditions and during liver regeneration, because cyclic AMP level has been observed to be altered during this process [17,18].

2. Experimental

Male Wistar rats (150 g average weight) were sham-operated or partially hepatectomised [19] to prepare adenylate cyclase and phosphodiesterase. 15 and 24 h after operation liver was taken and homogenised in 50 mM Tris HCl pH 7.0. Homogenate was centrifuged at 2000 × *g* for 15 min and pellet was washed three times with the same buffer. Adenylate cyclase was assayed in the pellet resuspended in 50 mM Tris (100 μg/100 μl). The first supernatant was centrifuged at 105 000 × *g* for 90 min. A fraction was used as such and a fraction was dialysed for 48 h against three changes of a solution containing 10 mM Tris, 2 mM $MgCl_2$ and 0.05 mM EGTA and against three additional changes of the same medium without EGTA. Both fractions were used for the phosphodiesterase assay. A portion of the soluble fraction was heated at 90°C for 3 min and was added to the incubation media of the enzymatic assays in order to test the possible activating action (100 μg protein/400 μl final volume of incubation).

As far as enzymatic assays are concerned adenylate cyclase was tested in a medium containing in 0.4 ml final volume, 50 mM Tris HCl, 10 mM theophilline, 3 mM ATP, 9 mM $MgSO_4$ and 100 μg enzyme. Further additions are indicated in tables. After 10-min

incubation at 37°C, the reaction was stopped by 2-min boiling; the cyclic AMP produced was measured according to the method of Gilman modified by Brown [20,21]. The phosphodiesterase was assayed with the method of Thompson and Appleman using 10^{-4} M cyclic AMP as substrate [22]. Proteins were estimated according to the method of Lowry [23].

3. Results and discussion

As far as adenylate cyclase is concerned, the basal activity appears to be higher 15 h after partial hepatectomy (table 1) as it has already been shown in isolated membrane [24]. Addition of EGTA at concentrations higher than 0.25 mM stimulates basal activity of regenerating liver. Enhanced stimulation is evident in presence of boiled supernatant. Such an enhancement does not appear to be modified when EGTA is added at concentrations ranging from 0.25 to 5 mM, whereas a clear inhibition is observed when added at 10 mM concentration.

In table 2 the results are shown concerning the addition of varying amounts of Ca^{2+} in presence of 10 mM EGTA. Enzymatic activity appears to be inhibited by Ca^{2+} at concentrations ranging from 7.5 to 20 mM; when the soluble fraction is present Ca^{2+} stimulates the activity at concentrations ranging from 2.5 to 7.5 mM; no effect is observed at higher concentrations.

In control animals, adenylate cyclase does not undergo any change in the above mentioned conditions. In table 3 the results are reported concerning the action of the soluble fraction from livers of partially hepatectomised rats on adenylate cyclase activity of sham operated animals. In these conditions no effect is exerted by Ca^{2+} or by soluble fraction.

No change in the activity of soluble high K_m phosphodiesterase is observed after 24 h from partial hepatectomy and no Ca^{2+} or soluble fraction dependence is evident (table 4).

These data show that 15 h after partial hepatectomy – at the onset of the S phase of cell cycle –

Tabl 1

Effect of EGTA, in the presence and in the absence of 100 μg boiled supernatant protein (SP), on adenylate cyclase. Activity is expressed as picomoles of cyclic AMP ± SD formed in 10 min per mg of particulate proteins. Number of experiments is indicated in parentheses. *P* has been evaluated by Student's *t* test

	15 h partially hepatectomised rats	15 h sham operated rats
Basal activity	120 ± 15	42 ± 18 $P<0.001$
+ 0.25 mM EGTA	112 ± 11 n.s.[a]	–
+ 0.7 mM EGTA	362 ± 55 $P<0.01$[a]	–
+ 1 mM EGTA	320 ± 81 $P<0.01$[a]	63 ± 20 n.s.[b]
+ 5 mM EGTA	381 ± 62 $P<0.01$[a]	34 ± 10 n.s.[b]
+ 10 mM EGTA	249 ± 17 $P<0.01$[a]	43 ± 18 n.s.[b]
+ SP	402 ± 78 $P<0.001$[a]	38 ± 10 n.s.[b]
+ SP + 0.25 mM EGTA	480 ± 0 n.s.[c]	–
+ SP + 0.7 mM EGTA	389 ± 64 n.s.[c]	38 ± 10 n.s.[d]
+ SP + 1 mM EGTA	300 ± 53 n.s.[c]	52 ± 8 n.s.[d]
+ SP + 5 mM EGTA	328 ± 44 n.s.[c]	60 ± 14 n.s.[d]
+ SP + 10 mM EGTA	124 ± 24 n.s.[c]	22 ± 2 n.s.[d]

[a] P with respect to basal activity of partially hepatectomised rats
[b] P with respect to basal activity of sham operated rats
[c] P with respect to basal activity in the presence of SP from partially hepatectomised rats
[d] P with respect to basal activity in presence of SP from sham operated rats

Table 2
Effect of varying concentrations of Ca^{2+} and 10 mM EGTA, in the presence or in the absence of boiled supernatant protein (SP), on adenylate cyclase. Activity is expressed as picomoles of cyclic AMP ± SD formed in 10 min per mg of particulate proteins; number of experiments carried out in duplicate are indicated in parentheses. *P* has been evaluated by Student's *t* test

	15 h partially hepatectomised rats	15 h sham operated rats
+ 10 mM EGTA (5)	249 ± 17	43 ± 18
+ 10 mM EGTA + 1.25 mM $CaCl_2$ (5)	210 ± 16 3 $P<0.5$[a]	35 ± 15 n.s.[b]
+ 10 mM EGTA + 2.5 mM $CaCl_2$ (5)	279 ± 33 n.s.[a]	33 ± 11.5 n.s.[b]
+ 10 mM EGTA + 5 mM $CaCl_2$ (5)	261 ± 40 n.s[a]	70 ± 16 n.s.[b]
+ 10 mM EGTA + 7.5 mM $CaCl_2$ (5)	105 ± 11.9 $P<0.001$[a]	36 ± 12 n.s.[b]
+ 10 mM EGTA + 10 mM $CaCl_2$ (5)	66 ± 19.1 $P<0.001$[a]	44 ± 12 n.s.[b]
+ 10 mM EGTA + 15 mM $CaCl_2$ (5)	38 ± 14 $P<0.001$[a]	56 ± 10 n.s.[b]
+ 10 mM EGTA + 20 mM $CaCl_2$ (5)	45 ± 22 $P<0.001$[a]	17 ± 3 $P<0.05$[b]
+ SP + 10 mM EGTA (5)	124 ± 24	22 ± 2
+ SP + 10 mM EGTA + 1.25 mM $CaCl_2$ (5)	–	–
+ SP + 10 mM EGTA + 2.5 mM $CaCl_2$ (5)	460 ± 20.3 $P<0.001$[c]	18 ± 7 n.s.[d]
+ SP + 10 mM EGTA + 5 mM $CaCl_2$ (5)	296 ± 65 $P<0.001$[c]	16 ± 45 n s.[d]
+ SP + 10 mM EGTA + 7.5 mM $CaCl_2$ (5)	224 ± 20 $P<0.05$[c]	38 ± 18 n.s.[d]
+ SP + 10 mM EGTA + 10 mM $CaCl_2$ (5)	204 ± 50 n.s.[c]	32 ± 8.5 n.s.[d]
+ SP + 10 mM EGTA + 15 mM $CaCl_2$ (5)	148 ± 50 n.s.[c]	21 ± 5 n.s.[d]
+ SP + 10 mM EGTA + 20 mM $CaCl_2$ (5)	83 ± 30 n.s.[c]	18 ± 7 n.s.[d]

[a] P with respect to activity in the presence of EGTA in partially hepatectomised rats
[b] P with respect to activity in the presence of EGTA in sham operated rats
[c] P with respect to activity in the presence of EGTA and SP in partially hepatectomised rats
[d] P with respect to activity in the presence of EGTA and SP in sham operated rats

Table 3
Effect of boiled supernatant protein (SP) from liver of 15 h partially hepatectomised rats on adenylate cyclase from liver of 15 h sham operated rats in the presence of EGTA and in the presence of EGTA and Ca^{2+}. Number of experiments is indicated in parentheses. Activity is expressed as pmoles/mg protein/10 min ± SD

Basal activity	42 ± 18
+ SP (5)	38 ± 10
+ SP + 0.25 mM EGTA (5)	43.2 ± 7
+ SP + 0.7 mM EGTA (5)	41.6 ± 6.0
+ SP + 5 mM EGTA (5)	44.8 ± 8.0
+ SP + 10 mM EGTA (5)	48.8 ± 9.2
+ SP + 10 mM EGTA + 1.25 mM $CaCl_2$ (6)	54 ± 11
+ SP + 10 mM EGTA + 2.5 mM $CaCl_2$ (6)	46.4 ± 9.5
+ SP + 10 mM EGTA + 5 mM $CaCl_2$ (6)	52.4 ± 13.2
+ SP + 10 mM EGTA + 7.5 mM $CaCl_2$ (6)	51.2 ± 11
+ SP + 10 mM EGTA + 10 mM $CaCl_2$ (6)	50.2 ± 10.5
+ SP + 10 mM EGTA + 15 mM $CaCl_2$ (6)	43 ± 8.3
+ SP + 10 mM EGTA + 20 mM $CaCl_2$ (6)	34 ± 5.2

Table 4
Effect of EGTA, Ca^{2+} and boiled supernatant protein (SP) on phosphodiesterase. Activity is expressed as nmoles AMP/mg protein/10 min±SD. *P* has been evaluated by Student's *t* test. Number of experiments is indicated in parentheses

	24 h partially hepatectomised rats	24 h sham operated rats
Basal activity	31.6 ± 6 n.s.	36.2 ± 2 n.s.
+ SP (6)	31 1 ± 3 n.s.	37.5 ± 4 n.s.
+ 1 mM EGTA (6)	34.8 ± 5 9 n.s.	35.4 ± 6 n.s
Dialysed basal activity	31.4 ± 4.9 n.s.	36.2 ± 3 n.s.
+ 1 mM EGTA (6)	38.1 ± 4.7 n.s.	35.3 ± 4 n.s.
+ 1 mM EGTA + 0.1 mM Ca^{2+} (6)	34.7 ± 2.5 n.s.	35 ± 2 n.s.
+ 1 mM EGTA + 1 mM Ca^{2+} (6)	36.6 ± 3.4 n.s.	37.4 ± 7 n.s.
+ 1 mM EGTA + 10 mM Ca^{2+} (6)	37.1 ± 7.4 n.s.	38.7 ± 4 n.s.
+ 1 mM EGTA + 10 mM Ca^{2+} + SP (6)	32 ± 4.5 n.s.	37.2 ± 2 n.s.

in parallel with the increase of cyclic AMP and of adenylate cyclase activity, this enzyme shows a dependence on the factors present in the soluble fraction. As to phosphodiesterase no Ca^{2+} dependence has been observed and this fact is confirmed by other authors [25,26]. It is not known whether the Ca^{2+} modulation and the action stimulating adenylate cyclase observed in soluble fraction are due to a Ca^{2+} binding specific protein, as it has already been shown for brain adenylate cyclase and phosphodiesterase.

Studies are being performed aiming to identify and characterize such a factor and to establish the importance of this control system during liver regeneration.

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