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HORMONE RESPONSIVENESS OF PLASMA MEMBRANE-BOUND ENZYMES IN NORMAL AND REGENERATING RAT LIVER

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Summary

The hormonal responsiveness of plasma membrane-bound enzymes (Na^+-K^+)-ATPase and adenylate cyclase has been investigated in normal and regenerating rat liver. (Na^+-K^+)-ATPase basal activity is not affected by surgery and only slightly affected by partial hepatectomy; its response to epinephrine and cyclic AMP is decreased only 15 h after hepatectomy. Adenylate cyclase activity of plasma membranes from untreated animals is stimulated by parathyroid hormone and thyroxine; partial hepatectomy increased basal activity as well as the stimulation exerted by the aforementioned hormones, when glucagon and epinephrine sensitivity is essentially unaltered.

The plasma membrane-linked formation of cyclic AMP and its hormone-dependent intracellular concentration modulation [1,2] lend themselves to a suitable experimental approach when the membrane itself is involved in some major physiological event like cell growth and differentiation [3—5].

In the present research, our interest has been focused on the hormone responsiveness of plasma membrane-bound enzymes (Na^+-K^+)-ATPase and adenylate cyclase at early stages of liver regeneration.

Previous research [6—8] has shown that (Na^+-K^+)-ATPase activity of rat liver plasma membrane is inhibited by epinephrine and cyclic AMP: this effect is essentially unaltered during the course of liver regeneration. Moreover, adenylate cyclase activity has been tested in the presence either of known hormonal activators like glucagon and epinephrine, or of hormonal substances like parathyroid hormone and thyroxine, which seem to be involved in the

process of liver regeneration [9—12]; the last two hormonal substances tested, have shown enhanced stimulation of enzymatic activity during regeneration.

Male Wistor rats (200 g average body weight) were used throughout this research and they were fed ad libitum. Partial hepatectomy was performed by the procedure of Higgins and Anderson [13] under ether anesthesia. Animals were sacrificed by cervical dislocation 4 or 15 h after surgical treatment. During this period they were fed normally.

Liver plasma membranes were isolated following the procedure of Ray [14,15] and their purity was assayed as reported previously [6].

(Na^+-K^+)-dependent ATPase activity was measured basically following the procedure of Bonting [16]. The experimental details have been already reported [6]. Incubation was carried out for 5 min at 37° C in a medium containing: membrane suspension (80—120 μg proteins), 92 mM Tris buffer (pH 7.5), 5 mM $MgSO_4$, 5 mM KCl, 0.1 mM EDTA, 4 mM ATP and the substances under investigation.

Adenylate cyclase activity was assayed in a medium containing, in a final volume of 0.4 ml, membrane suspension (80—120 μg proteins), 25 mM Tris buffer (pH 7.5), 4 mM $MgSO_4$; 5 mM aminophylline, 1 mM ATP and the substances under investigation. After 10 min at 37° C, reaction was stopped by boiling for 2 min and cyclic AMP produced was measured according to the procedure of Gilman [17], as modified by Brown et al. [18].

Protein was estimated by the method of Lowry et al. [19], as modified by Emmelot and Bos [20], using bovine serum albumin as a standard.

Glucagon, L-epinephrine bitartrate, L-thyroxine, parathyroid extract (263 U.S.P. parathyroid units per mg) were from Sigma. ATP (disodium salt) and cyclic AMP (adenosine 3′-5′ monophosphoric acid) were from Boehringer-Mannheim, Germany. [8-^{3}H]adenosine 3′-5′ monophosphoric acid, ammonium salt (30 Ci/nmol) was obtained from the Radiochemical Centre, Amersham, Bucks., U.K.

(Na^+-K^+)-ATPase activity of liver plasma membrane isolated from untreated, sham-operated and partially hepatectomized animals is reported in Fig.1. Control experiments show that enzyme activity decreases significantly 4 h after hepatectomy, returning to normal after 15 h, with a probability level of $P< 0.02$ with respect to sham-operated and untreated animals, and $P< 0.05$ with respect to 15 h hepatectomized animals; all other differences are statistically not significant. Epinephrine sensitivity, a feature already shown for (Na^+-K^+)-ATPase from rat liver plasma membrane [6,7] is decreased 4 h after surgery ($P< 0.02$) in sham-operated animals, returning to the normal response after 15 h; in partially hepatectomized animals, 4 h after surgery, response to epinephrine is the same as for sham-operated rats, but 15 h after surgery the response is significantly different ($P< 0.05$) with respect to sham-operated animals. Cyclic AMP dependence of (Na^+-K^+)-ATPase activity from liver membranes [6—8] is basically unchanged, except that 15 h after hepatectomy we have noticed a significant decrease ($P< 0.05$) of inhibition.

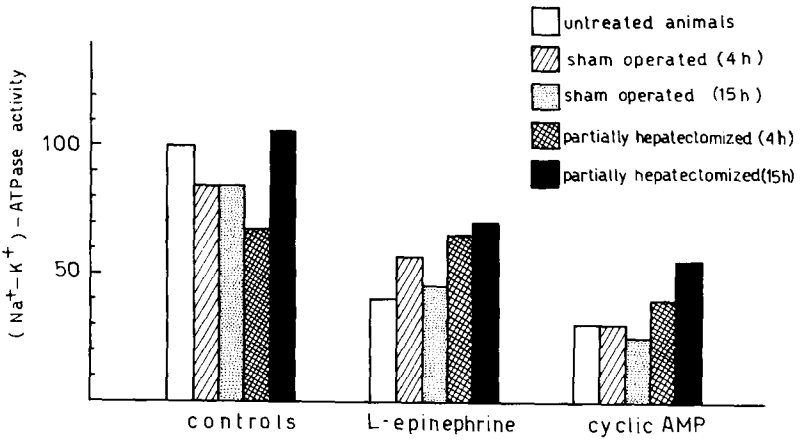

Fig.1. Results are reported as percentage of the basal activity of rat liver membranes isolated from untreated animals which was 1.08 ± 0.07 μmol P_i/mg protein per 5 min. The data are means of 6 triplicate experiments carried out on different membrane preparations. Standard error did not exceed 12% of the indicated value. *P* (see text) has been evaluated by the Student's *t*-test.

Table I shows adenylate cyclase activity of liver plasma membrane from untreated, sham-operated and partially hepatectomized animals in response to NaF and hormonal stimulation. Basal adenylate cyclase activity, which is in agreement with current literature [2,25], was not affected in liver membranes obtained from sham-operated animals, but it was significantly increased after partial hepatectomy. Enzyme activity of membranes isolated from untreated and sham-operated animals was normally stimulated by glucagon and NaF, but, for the latter substance, to a lower extent in sham-operated animals. In the case of partially hepatectomized animals, the normal sensitivity to the above-mentioned substances was retained; epinephrine response was sharply decreased or lost after surgical treatment. Adenylate cyclase response to parathyroid extract and thyroxine shows peculiar features: parathyroid extract stimulated adenylate cyclase activity in any condition, being more effective 4 h rather than 15 h after hepatectomy; thyroxine did not affect very much enzyme activity in the case of untreated and sham-operated animals, but strongly activated adenylate cyclase of membranes isolated from animals 4 h after partial hepatectomy, being only slightly effective 15 h after surgery.

Experiments carried out (Table II) to investigated a possible independent effect of parathyroid hormone and thyroxine on adenylate cyclase from normal liver membranes failed to show additive stimulation, suggesting that some interaction could take place at the membrane level. Conversely, both hormonal substances gave additional stimulation with glucagon. It should be remembered, in this context, that adenylate cyclase activity of a rat liver crude membrane preparation seems to be stimulated by parathyroid hormone and glucagon additively, as shown very recently by Moxley et al. [21].

TABLE I

HORMONAL RESPONSIVENESS OF LIVER MEMBRANE ADENYLATE CYCLASE FROM UNTREATED, SHAM-OPERATED AND PARTIALLY HEPATECTOMIZED RATS

Enzyme activity is reported as pmol cyclic AMP ± S.E./mg protein per 10 min; numbers in parentheses indicate duplicate experiments carried out on different membrane preparations. *P* has been evaluated by Student's *t*-test with respect to basal activity; with respect to basal activity of the correspondent sham-operated control (*); n.s., not significant. For experimental details, see text.

	Untreated animals	4h after operation		15h after operation	
		Sham operated	Partially hepatectomized	Sham operated	Partially hepatectomized
Basal activity	57 ± 4.3 (14)	54 ± 4 (4)	71 ± 8.1 (5) $P<0.05$ (*)	59 ± 2 (4)	158 ± 18 (4) $P<0.01$ (*)
NaF, 10 mM	725 ± 109 (10) $P<0.001$	382 ± 44 (4) $P<0.01$	713 ± 92 (4) $P<0.001$	320 ± 40 (4) $P<0.01$	969 ± 85 (3) $P<0.01$
L-Epinephrine, $1\cdot10^{-5}$ M	93 ± 15 (9) $P<0.001$	56 ± 4 (4) n.s.	98 ± 9.4 (5) $0.1>P>0.05$	123 ± 25 (3) $P<0.02$	139 ± 42 (4) n.s.
Glucagon, $1\cdot10^{-7}$ M	192 ± 28 (4) $P<0.05$	92 ± 14 (4) $P<0.05$	229 ± 37 (4) $P<0.001$	126 ± 3 (4) $P<0.001$	362 ± 81 (4) $P<0.05$
Parathyroid extract (units/ml)					
0.16	66 ± 8.1 (6) n.s.	64 ± 8 (4) n.s.	292 ± 11 (4) $P<0.001$	75 ± 2.3 (3) $P<0.05$	162 ± 12 (4) n.s.
1.6	180 ± 18 (6) $P<0.01$	128 ± 20 (4) $P<0.01$	235 ± 8 (4) $P<0.001$	130 ± 5 (3) $P<0.01$	209 ± 18 (3) n.s.
16	225 ± 41 (4) $P<0.001$	215 ± 15 (4) $P<0.001$	241 ± 20 (3) $P<0.001$	228 ± 19 (3) $P<0.001$	265 ± 25 (3) $P<0.02$
160	214 ± 35 (4) $P<0.001$	210 ± 14 (3) $P<0.001$	225 ± 21 (4) $P<0.001$	212 ± 18 (3) $P<0.001$	270 ± 34 (3) $P<0.05$
L-Thyroxine (M)					
$1\cdot10^{-7}$	65 ± 7.5 (4) n.s.	56 ± 2 (4) n.s.	234 ± 20 (4) $P<0.001$	82 ± 22 (3) n.s.	146 ± 4 (3) n.s.
$1\cdot10^{-6}$	67 ± 7.1 (5) n.s.	49 ± 1 (3) n.s.	211 ± 9.4 (3) $P<0.001$	90 ± 9.4 (3) $P<0.05$	138 ± 27 (3) n.s.
$1\cdot10^{-5}$	73 ± 10.7 (7) $P<0.05$	78 ± 15 (4) n.s.	268 ± 34 (4) $P<0.001$	82 ± 2 (3) $P<0.05$	205 ± 40 (3) n.s.
$1\cdot10^{-4}$	70 ± 8.8 (7) $P<0.05$	42 ± 4 (3) n.s.	298 ± 27 (4) $P<0.001$	88 ± 13 (3) $P<0.05$	196 ± 38 (3) n.s.

TABLE II

ADDITIVE EFFECTS OF PARATHYROID EXTRACT AND THYROXINE ON LIVER ADENYLATE CYCLASE FROM UNTREATED RATS

Enzyme activity is reported as pmol cyclic AMP ± S.E./mg protein per 10 min. Results are means of four duplicate experiments carried out on different membrane preparations. *P* has been evaluated by Student's *t*-test, with respect to control; with respect to the corresponding hormonal treatment in the absence of glucagon (*). For experimental details see text.

Treatment	Adenylate cyclcase activity	*P* value
None	58 ± 7	
Glucagon, $1 \cdot 10^{-7}$ M	250 ± 35	<0.001
Parathyroid extract, 16 units/ml	132 ± 20	<0.01
Thyroxine, $1 \cdot 10^{-4}$ M	81 ± 6.5	<0.05
Glucagon, $1 \cdot 10^{-7}$ M + Parathyroid extract, 16 units/ml	304 ± 28	<0.001 <0.01 (*)
Glucagon, $1 \cdot 10^{-7}$ M + Thyroxine, $1 \cdot 10^{-4}$ M	290 ± 30	<0.001 <0.001 (*)
Thyroxine, $1 \cdot 10^{-4}$ M + Parathyroid extract, 16 units/ml	122 ± 18	<0.01

(Na^+-K^+)-ATPase basal activity of rat liver plasma membrane is not affected by a surgical manipulation of the animal, and only slightly influenced by partial hepatectomy: this result is difficult to compare with previous observations carried out later on during regeneration and using a less purified enzyme preparation [22]. The hormonal response of (Na^+-K^+)-ATPase to the surgical manipulation is slightly affected in sham-operated animals, but definitely decreased after partial hepatectomy, particularly at 15 h. The enzyme response to cyclic AMP is not altered until 15 h after partial hepatectomy decreased sensitivity at this stage could be dependent on the second wave of cyclic AMP production during liver regeneration observed by MacManus and coworkers [11,23].

More dramatic results are obtained for adenylate cyclase where the basal response is markedly increased after hepatectomy and it seems to be very responsive, particularly at an early stage of regeneration, to the presence of parathyroid hormone and thyroxine; two hormones which are probably involved in the process of liver regeneration [9—12]. The increase of liver adenylate cyclase activity in partially hepatectomized animals, as well as its change in hormonal response, can be compared with similar results obtained for hepatic adenylate cyclase after chemically induced neoplasia [24,25].

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