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Characterization of purinoceptors present on human liver plasma membranes

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Using ATPα^{35}S as radioligand, we have detected the presence of specific purinoceptors on human liver plasma membranes. They are characterized by a K_d value of 0.19 μM and a B_{max} of 24 pmol/mg membrane protein. These purinoceptors belong to the P_{2Y} subclass as demonstrated by the high degree of similarity with rat liver purinoceptors, previously shown to be P_{2Y} [(1986) Biochem. J. 240, 367–371] and known to be involved in the control of liver glycogenolysis.

Purinoceptor; Glycogenolysis; (Human liver)

1. INTRODUCTION

As reviewed by Burnstock and Kennedy [1] and Gordon [2], extracellular ATP and ADP at micromolar concentrations can influence many biological processes. On the basis of extensive pharmacological studies, two subclasses of purinoceptors have been described: P_1-receptors are more sensitive to adenosine and AMP, positively linked to adenylate cyclase and blocked by methylxanthines; P_2-receptors are more specific for ATP and ADP, not antagonized by methylxanthines and not linked to adenylate cyclase. Burnstock and Kennedy [1] have proposed distinguishing P_2-purinoceptors via classification into two subtypes, designated P_{2X} and P_{2Y}. The P_{2X}-subtype shows a specific rank order of potency of pp[CH_2]pA $\approx$ p[CH_2]ppA $>$ ATP = 2-methylthio-ATP and is selectively desensitized by pp[CH_2]pA. The P_{2Y}-subtype displays a different rank order of potency (2-methylthio-ATP $\gg$ ATP $>$ pp[CH_2]pA $\approx$ p[CH_2]ppA) and is only weakly, if at all, desensitized by pp[CH_2]pA.

Rat liver glycogenolysis is clearly regulated by submicromolar concentrations of ATP and ADP [3–7]. These studies have shown that the purinoceptors involved belong to the P_2-class and initiate cAMP-independent, calcium-dependent glycogenolysis. Using ATPα^{35}S as radioligand, we [8] demonstrated the presence of specific high-affinity P_{2Y}-purinoceptors in rat liver. The present study shows that plasma membranes from human liver also possess P_{2Y}-purinoceptors very similar to those of rat liver and suggests therefore that in man, liver metabolism is possibly under purinergic control.

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Abbreviations: ATPαS, adenosine 5′-[α-thio]triphosphate; ATPα^{35}S, adenosine 5′-[α-[^{35}S]thio]triphosphate; ATPγS, adenosine 5′-[γ-thio]triphosphate; pp[CH_2]pA, adenosine 5′-[α,β-methylene]triphosphate; p[CH_2]ppA, adenosine 5′-[β,γ-methylene]triphosphate; p[NH]ppA, adenosine 5′-[β,γ-imido]triphosphate; Ap_3A, diadenosine triphosphate; Ap_4A, diadenosine tetraphosphate

2. MATERIALS AND METHODS

2.1. *Materials*

ATPαS, ATPα^{35}S and [^{3}H]dihydroalprenolol were purchased from Amersham International (England); reactive blue (a P_{2Y}-antagonist according to [9]) from Janssen (Beerse); and diadenosine triphosphate (Ap_3A) and tetraphosphate (Ap_4A) from Sigma (St. Louis, MO). These dinucleotides have been

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shown to increase glycogen phosphorylase in isolated rat hepatocytes [10] and we have confirmed this observation. We also checked that reactive blue antagonizes the glycogenolytic activity of ATP as tested in rat hepatocytes. The sources of other chemicals have been described in [3,8].

The computer program 'Enzfitter' was from Elsevier Biosoft (Cambridge, England).

2.2. *Methods*

Rat liver plasma membranes were isolated as in [11] with minor modifications [8]. Tumor-free non-cirrhotic human liver samples were obtained from patients undergoing hemihepatectomy for benign or malignant tumors. Approval was obtained from the ethical commission of the Faculty of Medicine. Tissue samples were frozen in liquid nitrogen and kept at −80°C. For preparation of liver plasma membranes, liver was first powdered in a mortar under liquid N_2. The subsequent procedure was according to [12].

We verified that adenylate cyclase of plasma membrane preparations was stimulated by glucagon (3–4-fold) and NaF (6-fold). We also checked that for human liver plasma membranes the binding of [^{3}H]dihydroalprenolol (K_d = 0.9 nM and B_{max} = 76 fmol/mg membrane protein) was similar to that described in [12].

ATPα^{35}S binding to purified plasma membranes and determination of the dissociation constants of the ATP analogues by competition experiments were performed as in [8].

3. RESULTS AND DISCUSSION

Fig.1 illustrates the time-dependent association and dissociation of ATPα^{35}S with purified human liver plasma membranes. The association is rapid, equilibrium being reached in less than 1 min, and reversible, since the addition of excess ATP results in rapid and fairly complete displacement of the radioligand.

Steady-state binding measurements were performed after 1 min incubation. The dose-dependent binding of ATPα^{35}S to purified human liver membranes is shown in fig.2. The binding pattern cannot be described adequately by a simple Michaelis-Menten equation as is also illustrated by the curvilinear Scatchard plot. Computer analysis

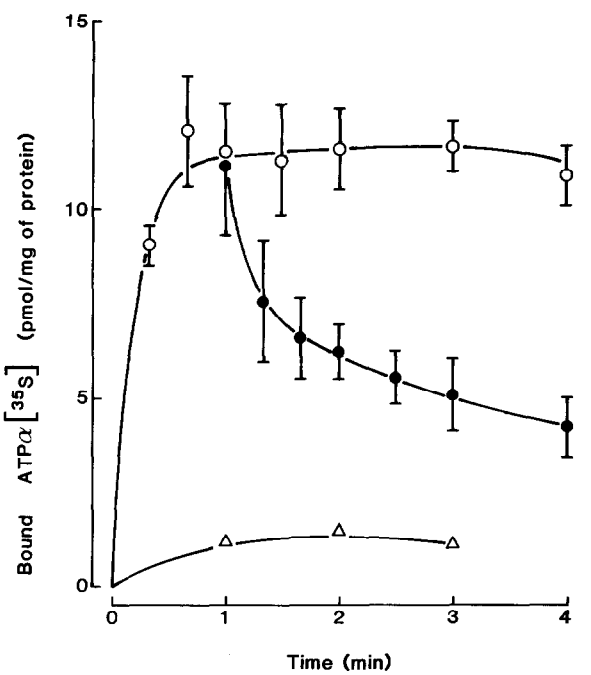

Fig.1. Association-dissociation pattern for the binding of ATPα^{35}S to human liver plasma membranes. Plasma membranes (0.6 mg protein/ml) were incubated with 0.12 μM ATPα^{35}S; 1 min later excess ATP (1 mM) was added. Total [(○) association and (●) dissociation] and non-specific (△) binding are plotted vs time. Values are means ± SE (where scale permits) from 3 independent experiments.

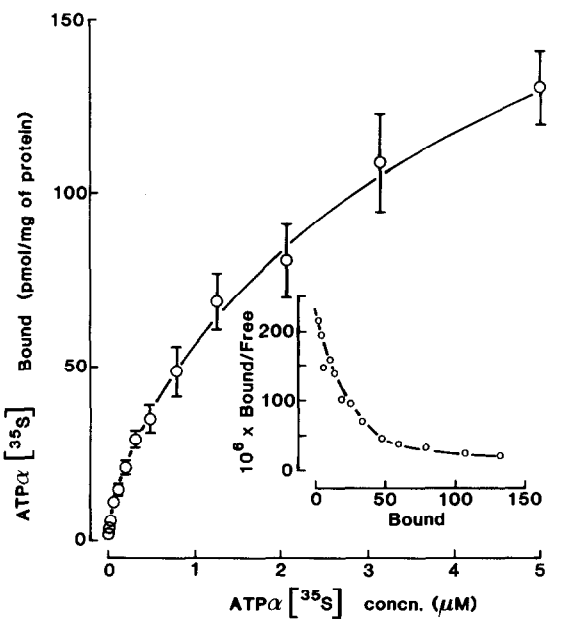

Fig.2. Dose dependence of specific ATPα^{35}S binding to purified human liver plasma membranes. Liver plasma membranes (0.6 mg protein/ml) were incubated with the indicated concentrations of ATPα^{35}S. Specific binding was measured after 1 min; values given as means ± SE (where scale permits) for 6 independent observations. (Inset) Scatchard plot. Computer analysis reveals that the data can be accounted for on the basis of the existence of a class of high-affinity binding sites and of a non-saturable component. For the high-affinity site, K_d (± SE) and B_{max} (± SE) are 0.19 ± 0.09 μM and 24 ± 9 pmol/mg protein, respectively.

of the data reveals that the binding pattern can be considered as being the consequence of two independent components. One represents saturable binding, characterized by a K_d of 0.19 μM and a maximal capacity of 24 pmol/mg protein. The other, observed at higher concentrations of ATPα^{35}S, can be considered as non-saturable binding. This situation is almost identical to that observed previously with rat liver membranes where the saturable high-affinity sites are characterized by a K_d value of 0.11 μM and a B_{max} of 30 pmol/mg membrane protein [8].

By using several ATP analogues and freshly prepared rat hepatocytes, we have obtained evidence that, in rat liver, the high-affinity binding sites correspond to the receptors mediating the activation of glycogen phosphorylase [8]. We did not have human hepatocytes readily available to compare the biological effects of ATP analogues with their binding affinities. Therefore, for a series of analogues, we determined the binding affinities for human liver plasma membranes in comparison with those for rat liver plasma membranes. Table 1 lists pK_d values characterizing the affinities of 10 different agonists and of one antagonist (reactive blue) for human and rat liver plasma membranes. Table 1 also contains the pK_d values that we determined previously for the binding of some analogues to intact rat hepatocytes [8]. The latter pK_d values correlate well (r = 0.933, P = 0.0005) with those determined in the present article with liver plasma membranes, indicating that the plasma membrane receptors correspond to those found on intact hepatocytes. The data in table 1, as shown also in fig.3, show that the pK_d values determined with human plasma membranes correspond very closely (r = 0.96, P = 0.001) with those for rat liver plasma membranes, strongly suggesting that human and rat liver possess the same class of purinoceptors.

Table 1

Comparison of the pK_d values of different analogues for rat and human liver plasma membranes

Analog	Plasma membranes		Cells[a] (rat)
	Rat	Human	
(1) ATPγS	6.9 ± 0.1 (3)	6.3 ± 0.1 (4)	5.8
(2) ATP	6.6 ± 0.2 (4)	6.7 ± 0.2 (4)	5.7
(3) ADP	6.2 ± 0.2 (5)	6.5 ± 0.2 (5)	6.1
(4) p[NH]ppA	5.2 ± 0.6 (2)	4.8 ± 0.6 (6)	4.8
(5) Reactive blue	5.1 ± 0.2 (3)	4.9 ± 0.1 (4)	
(6) pp[CH_2]pA	4.9 ± 0.1 (3)	4.3 ± 0.3 (6)	3.7
(7) p[CH_2]ppA	4.9 ± 0.1 (3)	4.5 ± 0.3 (5)	4.9
(8) GTP	4.8 ± 0.3 (5)	4.3 ± 0.4 (3)	4.6
(9) Ap_4A	4.4 ± 0.1 (2)	3.6 ± 0.1 (2)	
(10) Ap_3A	4.5 ± 0.3 (4)	3.4 ± 0.2 (3)	
(11) NADP	2.5 ± 0.5 (4)	2.0 ± 0.1 (2)	3.3

[a] From [8]

Dissociation constants for binding were calculated from data obtained in competition experiments with ATPα^{35}S, as in [8], using a K_d value for ATPα^{35}S of 0.19 μM and are expressed in terms of pK_d ($-\log K_d$) ± SD (n)

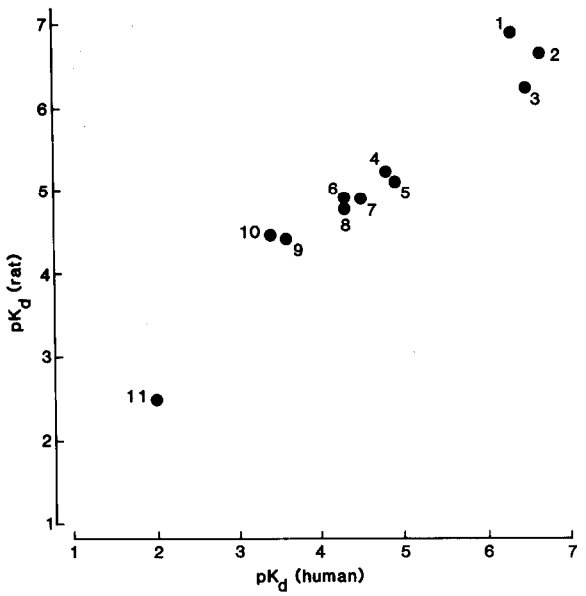

Fig.3. Correlation between pK_d values of different analogues for human and rat liver plasma membranes. Numbers correspond to the analogues listed in table 1 (r = 0.9785, P = 0.0001).

4. CONCLUSION

This study clearly demonstrates the presence of specific purinoceptors on human liver plasma membranes. Binding is rapid, essentially reversible and dose-dependent. The fact that human and rat liver purinoceptors show very similar characteristics suggests that in man ATP and ADP might also control liver metabolism through interaction with P_{2Y}-purinoceptors.

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