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Characterization of the α_1-adrenoceptors of dog liver: predominance of the α_{1A}-subtype

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Abstract

Using dog liver membranes we observed that [^{125}I]HEAT ((±)-β-([^{125}I]iodo-4-hydroxyphenyl)-ethyl-aminomethyl-tetralone) binds with high affinity (K_D 97 pM) to a discrete number of sites (B_{max} 40 fmol/mg protein) with the pharmacological characteristics expected for α_1-adrenoceptors. Such sites were inactivated by pretreatment with chloroethylclonidine. Binding competition experiments indicated the following order of potency: (a) for agonists: oxymetazoline > epinephrine ≥ norepinephrine > methoxamine and (b) for antagonists: WB4101 ≥ 5-methyl-urapidil = prazosin ≥ benoxathian ≥ (+)-niguldipine > phentolamine. Northern analysis indicated that total RNA isolated from dog liver hybridized with an α_{1c} selective probe (bovine brain). The orders of potency for agonists and antagonists, their K_i values and the Northern analysis suggest that dog liver expresses α_{1A}-adrenoceptors.

Keywords: α_1-Adrenoceptor subtype; Liver; Hepatocyte membrane; α_{1A}-Adrenoceptor

1. Introduction

Current evidence indicates that α_1-adrenoceptors constitute a heterogeneous family of receptors. Initially, the existence of two subtypes, the α_{1A}- and the α_{1B}-adrenoceptors, was suggested on a pharmacological basis (Morrow and Creese,1986; Minneman, 1988). Molecular biological techniques have allowed cloning and expression of three receptors: i.e. α_{1b} (Cotecchia et al., 1988) α_{1c} (Schwinn et al., 1990) and α_{1d} (Lomasney et al., 1991; Perez et al., 1991) α_1-adrenoceptors. It has been difficult to establish the relatioship between pharmacolgically defined (upper case) and cloned (lower case) α_1-adrenoceptors (Bylund et al., 1994). Current evidence indicates the following: (1) there is a close agreement between the pharmacological properties of the expressed α_{1b} cloned receptor with those recognized for the pharmacologically defined α_{1B}-adrenoceptor, i.e., the α_{1b} clone seems to correspond to the α_{1B}-adrenoceptor; (2) the α_{1d} clone seems to represent a novel subtype (i.e., the α_{1D}-adrenoceptor); (3) most current evidence suggests that the α_{1c} clone may correspond to the α_{1A}-adrenoceptor (Bylund et al., 1994; Forray et al., 1994; Rokosh et al., 1994).

However, considerable variation in the subtype of α_1-adrenoceptors expressed in liver cells of different species has been observed (García-Sáinz et al., 1992a). Thus, livers from rats (Han et al., 1987; García-Sáinz et al., 1992a; García-Sáinz et al., 1994), mice, hamsters (García-Sáinz et al., 1994) and chickens (Gutiérrez-Venegas and García-Sáinz, 1993) express α_{1B}-adrenoceptors whereas rabbit hepatocytes express α_{1A}-adrenoceptors (García-Sáinz et al., 1992a; Taddei et al., 1993). Guinea pig liver cells also express α_{1A}-adrenoceptors (García-Sáinz et al., 1992a; García-Sáinz et al., 1992b; García-Sáinz and Romero-Avila, 1993). There are clear differences in sensitivity to chloroethylclonidine between these α_{1A}-adrenoceptors (García-Sáinz et al., 1992a).

No detailed characterization of the α_1-adrenoceptor subtype present in dog liver has been reported. We undertook this task and our results suggest that dog liver mainly expresses α_{1A}-adrenoceptors.

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2. Materials and methods

(−)Epinephrine, (−)norepinephrine, oxymetazoline and prazosin were obtained from Sigma Chemical Co. Benoxathian, 5-methyl-urapidil, chloroethylclonidine, and WB 4101 were from Research Biochemicals. Phentolamine (Ciba-Geigy), methoxamine (Burroughs Wellcome) and (+)-niguldipine (Byk Gulden) were generous gifts from the indicated pharmaceutical companies. [^{125}I]HEAT ((±)-β-([^{125}I]iodo-4-hydroxyphenyl)-ethyl-aminomethyl-tetralone) (2200 Ci/mmol) was obtained from New England Nuclear.

Partially purified liver plasma membranes, from mongrel dogs were obtained by the method of Neville (1968) up to step 11. Membranes were washed and resuspended in 50 mM Tris, 10 mM $MgCl_2$, pH 7.5; aliquots of the membrane preparation were frozen and stored under liquid nitrogen until used (usually within 2 weeks), without any noticeable change in receptor binding. Binding studies were performed by incubating membranes (100 μg) in 50 mM Tris, 150 mM NaCl, 5 mM EDTA with the radioactive ligand, alone or with the indicated agents. Incubations were in a total volume of 0.25 ml for 60 min in a water bath shaker at 25°C. At the end of the incubation, 5 ml of ice-cold buffer were added to the membrane suspension, and the membranes were immediately filtered on GF/C filters and washed 3 times (5 ml each time) with the same buffer. Filters were presoaked with 0.3% polyethylenimine to decrease non-specific binding to the filter. Saturation experiments were performed with concentrations in the range of 5–400 pM [^{125}I]HEAT and binding competition studies with 100–200 pM of the radioactive ligand (50–60% receptor occupation). Non-specific binding was evaluated in the presence of 10 μM phentolamine; specific binding represented 70–80% of the total binding at the K_D. Binding saturation and competition data were analyzed using the EBDA and LIGAND (Munson and Rodbard, 1980) programs (Biosoft-Elsevier). K_i values were calculated according to Cheng and Prusoff (1973). Protein was quantified by the method of Lowry et al. (1951), using bovine serum albumin as standard.

Table 1
Binding parameters from [^{125}I]HEAT saturation experiments with dog liver membranes

Treatment	K_D (pM)	B_{max} (fmol/mg protein)
None (3)	97±15	40±5
Pre-incubated alone (4)	132±17	42±4
Pre-incubated with CEC (4)	111±30	17±3

Results are the means±S.E.M. with the number of determinations with different membrane preparations indicated in parentheses. CEC, chloroethylclonidine.

Total RNA was obtained by the method of Chomcynski and Sacchi (1987) with minor modifications. Northern analysis was performed using standard high stringency conditions as described before (García-Sáinz et al., 1992a). The probes were as follows: the 0.85 Kb BamHI fragment of rat α_{1d} cDNA (Lomasney et al., 1991), the 0.81 Kb EcoRI-BamHI fragment of hamster α_{1b} cDNA (Cotecchia et al., 1988) and the 0.95 Kb BglII fragment of bovine α_{1c} (Schwinn et al., 1990).

3. Results

As has been shown in many other systems (Bylund, 1987), binding of [^{125}I]HEAT to dog liver membranes was rapid, saturable and reversible. A representative saturation isotherm is presented in Fig. 1. The Rosenthal (1967) transformation of the saturation isotherms resulted in straight lines consistent with the binding of this ligand to a homogeneous class of binding sites. A relatively small number of binding sites, with high affinity for the radioligand, was observed (Table 1). Such affinity was within the range observed for α_1-adrenoceptors in several models (Bylund, 1987).

The sensitivity of the α_1-adrenoceptors of dog liver membranes to chloroethylclonidine was tested as follows: membranes were preincubated with 10 μM chloroethylclonidine for 15 min at 37°C; after this treatment, the membrane preparations were diluted with cold buffer and washed twice by centrifugation-resuspension. Treatment with chloroethylclonidine resulted in a marked decrease (55–75%) in receptor number with no major change in affinity for the radioligand (Fig. 1 and Table 1). Membrane preincubation with 100 μM chloroethylclonidine abolished [^{125}I]HEAT specific binding.

Binding competition studies indicated the following orders of potency for agonists: oxymetazoline > epinephrine ≥ norepinephrine > methoxamine. For antagonists the potency order was: WB4101 ≥ 5-methyl-urapidil = prazosin ≥ benoxathian ≥ (+)-niguldipine > phentolamine. Representative binding competition plots are presented in Fig. 2 and the K_i values and competition slopes are presented in Table 2. It is important to emphasize that the binding sites studied had high affinities, i.e., close to 1 nM, for all the antagonists tested except phentolamine. The slopes of the competition curves were in the range of 0.60–0.75 for agonists and 0.75–0.95 for antagonists (Table 2). LIGAND analysis of the antagonist competition curves indicated that no improvement in the goodness of the fit was obtained with two-state fits as compared to one-state fits, except in one experiment with WB4101 as competing ligand (K_H 0.4 nM, K_L 35 nM, R_H 88%, R_L 12%).

Northern analysis was performed with total RNA,

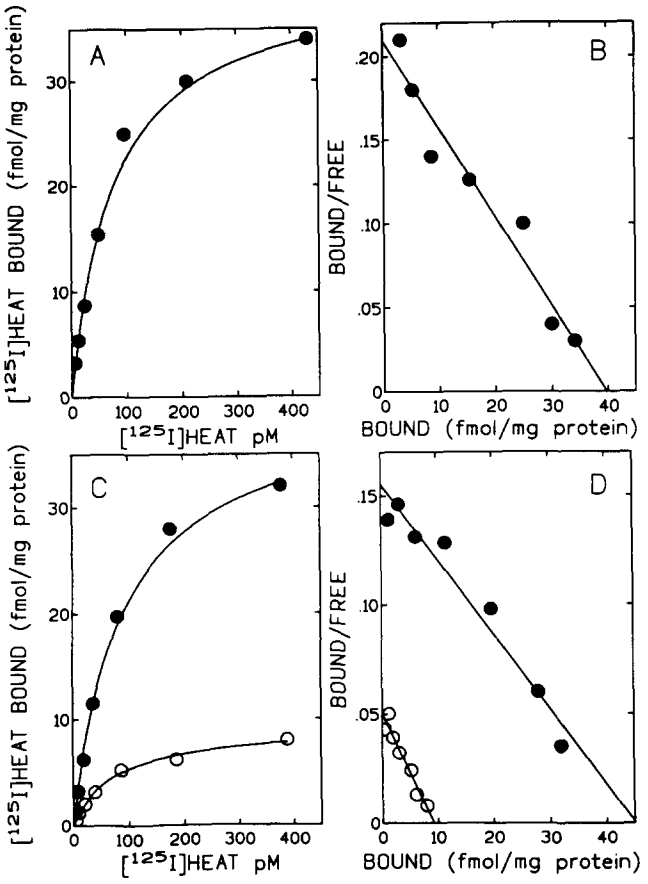

Fig. 1. [^{125}I]HEAT saturation isotherm for dog liver membranes and effect of chloroethylclonidine. Upper panels: plotted are the specific binding (A) and the Rosenthal analysis (B). The figure is representative of three experiments with different membrane preparations. Lower panels: membranes were preincubated in the absence (filled circles) or presence (open circles) of chloroethylclonidine; after this treatment the membranes were extensively washed. Plotted are the specific binding (C) and the Rosenthal analysis (D). The figure is representative of four experiments using different membrane preparations.

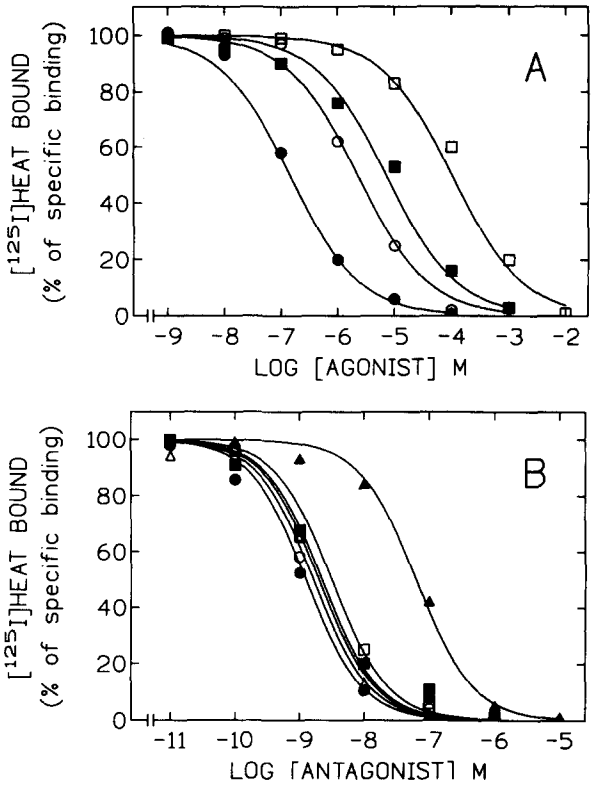

Fig. 2. Binding competition experiments with dog liver membranes. Panel A, the following agonists were used: oxymetazoline, filled circles; epinephrine, open circles; norepinephrine, filled squares; methoxamine, open squares. Panel B, the following antagonists were used: WB4101, filled circles; prazosin, open circles; 5-methyl-urapidil, filled squares; benoxathian, open squares; (+)-niguldipine, open triangles; phentolamine, filled triangles. The figures are representative of four to seven experiments with different membrane preparations.

extracted from dog liver, using the α_{1b}, α_{1c} and α_{1d} probes. Hybridization was clearly observed with the α_{1c} probe (Fig. 3); if the filters were exposed for long time some weak signal was also observed with the α_{1B} probe.

4. Discussion

It is well known that catecholamines play a major role in modulating hepatic metabolism; both α- and β-adrenoceptors mediate the actions of catecholamines. α_1-Adrenoceptors are particularly important and abundant in the liver of birds and small rodents, such as rats, mice and hamsters, whereas β-adrenoceptors predominate in other vertebrates (Sulakhe et al., 1988; Gutierrez-Venegas and García-Sáinz, 1993; García-Sáinz et al., 1994). In dogs, there is evidence that the glycogenolytic response to catecholamines is mainly mediated via β-adrenoceptors (Mayer et al., 1961; Newton and Hornbrook, 1972); however, α_1-adrenoceptors seem also to participate in the effects

Table 2
Binding parameters derived from the competition by agonists and antagonists for [^{125}I]HEAT binding sites in dog liver membranes

	K_i (nM)	Slope
Agonists		
Oxymetazoline (4)	62 ± 24	0.75 ± 0.02
(−)Epinephrine (4)	2630 ± 900	0.60 ± 0.08
(−)Norepinephrine (4)	2937 ± 734	0.70 ± 0.09
Methoxamine (4)	89935 ± 13975	0.72 ± 0.10
Antagonists		
WB4101 (6)	0.35 ± 0.04	0.82 ± 0.07
5-Methyl-urapidil (6)	0.73 ± 0.16	0.84 ± 0.04
Prazosin (7)	0.63 ± 0.10	0.85 ± 0.10
Benoxathian (5)	1.30 ± 0.55	0.89 ± 0.13
(+)-Niguldipine (7)	2.18 ± 0.50	0.80 ± 0.10
Phentolamine (4)	45.00 ± 6.00	0.95 ± 0.06

Results are the means ± S.E.M. with the number of determinations with different membrane preparations indicated in parentheses.

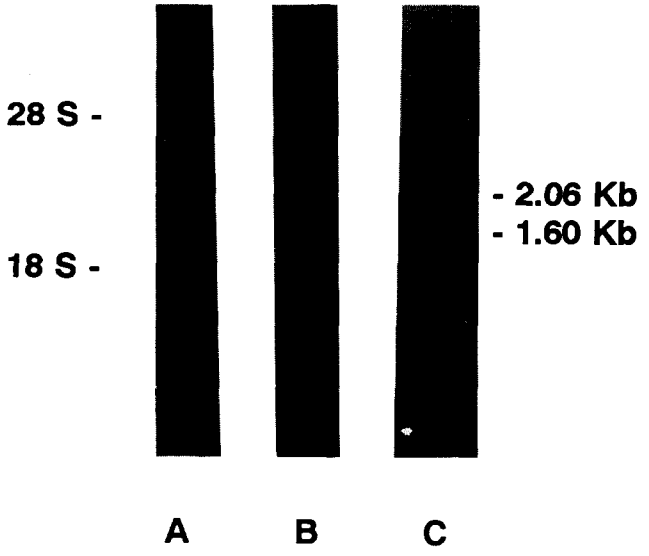

Fig. 3. Northern analysis. Total RNA was hybridized with the α_{1d} (lane A), α_{1b} (lane B) and α_{1c} (lane C) probes. This autoradiogram is representative of three experiments using different preparation of RNA, with identical results.

(Garceau et al., 1985; Maroto et al., 1993). Certainly, much more information is required on the role that these adrenoceptors play in modulating hepatic metabolism in these species.

To the best of our knowledge, the adrenoceptors present in dog liver have not been previously characterized. In the present work, we show that, using [^{125}I]HEAT, a discrete number of sites with the characteristics expected for α_1-adrenoceptors were detected.

The following data suggest that the majority of the α_1-adrenoceptors present in liver membranes belong to the α_{1A}-subtype. Firstly, the K_i values for agonists were within the ranges observed for the cloned receptor (α_{1c}) and membranes from rabbit liver (Schwinn et al., 1990, 1992; Taddei et al., 1993 and unpublished data from our laboratory). Secondly, the Northern analysis. Regarding antagonists, the relatively high affinity for WB4101 and 5-methyl-urapidil also suggests that these receptors belong to the α_{1A}-subtype. Comparison of the K_is for the other selective antagonists, (+)-niguldipine and benoxathian, is more complex. With the cloned α_{1c} receptor, K_i values of 500 nM for benoxathian and 80 nM for (+)-niguldipine were obtained (Schwinn and Lomasney, 1992). In contrast, a K_i of 5.5 nM was reported for the adrenoceptor in rabbit liver membranes (Taddei et al., 1993). We have observed, using rabbit liver membranes, K_is of 1–3 nM for both benoxathian and (+)-niguldipine (unpublished data). The reason for such differences in K_i values between the cloned receptor and that expressed in rabbit liver is unknown. α_1-Adrenoceptors of dog liver membranes have K_i values for these antagonists very similar to those of membranes from rabbit liver; their sensitivity to chloroethylclonidine is also similar (García-Sáinz et al., 1992a).

In summary, our data suggest that the majority of the α_1-adrenoceptors present in dog liver membranes belong to the α_{1A}-subtype. Some heterogeneity possibly exists in this tissue, as evidenced by the Northern analysis and the slopes of the binding competition studies. However, available data suggest that such receptors, probably of the α_{1B}-subtype, are a very small fraction of the α_1-adrenoceptors present in liver cells.

Our data also indicate that the receptor subtype present in a given tissue varies considerably among different species and that one should be aware of the enormous risks of extrapolating data from one species to another. It is, therefore, clear that much more work is required in this field to define receptor subtypes in specific tissues and cells and their physiological roles.

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