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EVIDENCE FOR HETEROGENEOUS DISTRIBUTION OF α_1, α_2- AND β-ADRENERGIC BINDING SITES ON RAT-LIVER CELL SURFACE

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Fractionation of preparations of rat-liver membranes on linear sucrose gradients revealed different profiles for the binding of α_1-, α_2- and β-adrenergic radioligands. The peaks of binding activities of [^{3}H]prazosin and [^{3}H]epinephrine were clearly separated from those of [^{3}H]yohimbine and [^{125}I]iodocyanopindolol which appeared at lower sucrose densities. Enzyme marker activities in the sucrose subfractions indicated the presence of plasma membranes in all of the subfractions. Furthermore, the binding peaks of the various adrenergic radioligands cannot be correlated with the presence of membranes derived from microsomes, lysosomes or Golgi apparatus. Pretreatment of rat livers with concanavalin A, in order to prevent the fragmentation of the plasma membranes during isolation, resulted in the shift of the binding of [^{3}H]yohimbine and [^{125}I]iodocyanopindolol to sucrose-gradient subfractions of higher densities, clearly separate from fractions containing microsomes and Golgi apparatus. There was no distinct separation of the binding peaks of prazosin, yohimbine, and cyanopindolol in sucrose-gradient subfractions from concanavalin A-pretreated livers. These results are consistent with the hypothesis that α_1-, α_2-, and β-adrenergic binding sites are associated with plasma membranes, and are heterogeneously distributed on the rat-liver cell surface.

Introduction

Binding of catecholamines to both α- and β-adrenergic receptors activates glycogenolysis and gluconeogenesis in rat liver [1,2]. In adult fed rats, epinephrine-mediated activation of glycogenolysis was shown to be through interaction with α_1-adrenergic receptors [3,4]. Mobilization of intracellular calcium ion is thought to be involved in the α_1-adrenergic action [5]. The activation of glycogenolysis via the β-adrenergic-adenylate cyclase system is minor in these animals. In young male and adult female rats, however, β-adrenergic activation of glycogenolysis may be more important [6,7]. Adrenalectomy [8], thyroidectomy [9] and fasting [10] have been shown to increase β-adrenergic activation of glycogenolysis. The physiological role of the α_2-adrenergic receptors is still unclear [11,12].

In the livers of adult male rats, 24-h fasting causes a decrease in the number of binding sites for both α_1- and α_2-adrenergic radioligands, concomitant with a 2-fold increase in the number of β-adrenergic binding sites [10]. In rat liver slices [13] and in hepatocytes primary cultures [14] the β-adrenergic receptors could be desensitized by prolonged incubation with β-adrenergic agonists, whereas no α_1-agonist-mediated desensitization properties were observed for the α_1-adrenergic receptors [15,16]. These differences in the observed patterns of receptors' turnover and desensitization (and probably internalization) may point to

organizational differences in the distribution of the various adrenergic receptors on the cell plasma membranes. These membranes have been divided into the bile-canalicular, the lateral and the blood sinusoidal regions on biochemical and physiological bases [17,18]. Most of the work on hepatic adrenergic receptor binding has nonetheless been carried out using a preparation rich in the bile-canalicular regions [19–22]. The possible heterogeneous distribution of the binding sites might have been thus obscured.

We have used both the methods of Song et al. [20] and Touster et al. [21] to isolate different fractions of plasma membranes for the purpose of investigating possible differences in the distribution of various adrenergic receptors on the cell surface.

Methods

[^{3}H]Prazosin (18.0 Ci/mmol), [^{3}H]yohimbine (35 Ci/mmol), and [^{125}I]iodocyanopindolol (2200 Ci/mmol) were from New England Nuclear. UDP-[^{14}C]galactose (uniformally labeled, 300 μCi/mmol) was from Amersham and [α-^{32}P]ATP (25 Ci/mmol) was from ICN. The sources of other chemicals have been reported previously [3,10,23–25].

Rat-liver plasma membranes prepared according to Song et al. [20] were obtained as previously described [10,23]. In the present paper, this preparation will be called the Song preparation. Plasma membranes were also prepared using the procedure described by Touster et al. [21] by the fractionation of what they labeled the microsomal fraction. The membranes obtained by this method will be called the Touster membranes. This membrane fraction was further subfractionated as follows:

(1) The fraction was suspended in 6 ml of 0.25 M sucrose/0.005 M Tris buffer (pH 8.0) (buffer I) and then layered on top of a discontinuous sucrose gradient (18 ml, 49%, w/v in 0.005 M Tris (pH 8.0) (buffer II) and 14 ml, 37%, w/v in buffer II) and then centrifuged at 78 000 × g for 3 h. The fraction called by Touster et al. [21] as the microsomal-light appeared at the interface of the top two layers.

(2) The pellet was suspended in buffer I and layered on top of a linear sucrose gradient (8–40%, w/v) and then centrifuged at 78 000 × g for 3 h. The polypropylene centrifuge tubes were then punctured and 2 ml fractions were collected.

Determination of marker enzyme activities. 5'-Nucleotidase (EC 3.1.3.5) was determined by the method of Avruch and Wallach [26] using [^{3}H]AMP in Tris buffer (pH 8.0). Glucose-6-phosphatase (EC 3.1.3.9) was determined by the method described by Baginski et al. [27]. Acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) were assayed according to the method described by Walter and Schutt [28] using 4-nitrophenylphosphate as a substrate. Acid phosphatase activity was determined in citrate buffer (pH 4.8) and alkaline phosphatase in 0.1 M diethanolamine (pH 9.8) containing 1.5 mM magnesium chloride and 0.1 mM zinc chloride [29]. UDP-galactose: *N*-acetylglucosamine galactosyltransferase (EC 2.4.1.38) activity was determined by the method described by Fleisher and Fleisher [30] and modified by Bergeron et al. [31]. Adenylate cyclase (EC 4.6.1.1) was determined by the method of Krishna et al. [32] in a reaction mixture (200 μl) containing 50 mM glycylglycine (pH 7.5), 1 mM 3-isobutylxanthine, 5 mM magnesium chloride, 0.3 mM MgATP, [α-^{32}P]ATP ($(1–2)\cdot 10^6$ cpm), 2 mM purified creatine phosphate, creatine kinase (100 μg/ml), myokinase (100 μg/ml), adenosine deaminase (5 U/ml) and 1 mM cyclic AMP (cAMP). Reactions were carried out for 5 min at 37°C. The reactions were started by addition of membranes and were stopped by the zinc acetate/sodium carbonate precipitation method. Labeled cAMP was purified as described by Salomon et al. [33].

Receptor binding assays. Binding assays were performed as described previously [10,23]. The definition of specific binding to the various sites as well as the verification of the binding data as binding to biologically relevant sites have been given in previous reports [10,23]. The specific-to-total binding ratios for the radioligands [^{3}H]epinephrine, [^{3}H]prazosin, [^{3}H]yohimbine and [^{125}I]iodocyanopindolol were, 0.7–0.8, 0.8–0.95, 0.7–0.8 and 0.7–0.8, respectively. The specific-to-total binding ratio was dependent on the radioligand concentrations and independent of the degree of membrane purification.

Protein was determined by the method described by Lowry et al. [34] using bovine serum albumin as standard.

Results

Binding of various adrenergic radioligands to the two different plasma membrane preparations

Table I shows the binding of the α_1-adrenergic antagonist prazosin (4 nM), the adrenergic agonist epinephrine (75 nM), the α_2-adrenergic antagonist yohimbine (10 nM), and the β-adrenergic antagonist cyanopindolol (0.2 nM) to Song membranes and to the microsomal-light fraction prepared as described in Methods. It is seen that the specific binding per mg protein of prazosin and epinephrine to Song membranes is greater than that to the microsomal-light of Touster. In contrast, specific binding per mg protein of yohimbine and cyanopindolol is less in the Song membranes than in the microsomal-light membrane fraction of Touster. The differences in the specific binding per mg protein between the two preparations are as follows: prazosin and epinephrine binding decreased by 35–40%, whereas binding of yohimbine and cyanopindolol increased by 3–4-fold in the Touster microsomal-light membranes as compared to binding in the Song membranes. 5′-Nucleotidase specific activity (per mg protein) is not different in the two preparations, whereas a 3–4-fold increase in the activity of glucose-6-phosphatase is observed in the microsomal-light membranes over that observed in the Song preparations (data not shown). These results are consistent with the presence of plasma membranes in the microsomal-light preparation which has been enriched in microsomes as expected [22].

Experiments were carried out to determine the specific binding of the α_2-adrenergic antagonist yohimbine to the microsomal-light membranes as a function of its concentration. Scatchard analysis [35] of the data yielded an association constant (K_d) of 19 nM (range of 17–21 nM) and total number of binding sites equivalent to 230 fmol/mg protein (range of 210–300 fmol/mg protein) for yohimbine. We have previously reported similar K_d values (21.0 ± 4.0) for yohimbine binding to the Song membranes [10]. These results indicate that the two populations of yohimbine-binding sites in the two preparations exhibit similar binding properties and that the increased binding in

TABLE I

BINDING OF VARIOUS ADRENERGIC RADIOLIGANDS TO SONG AND MICROSOMAL-LIGHT MEMBRANES

The definition of specific binding and incubation conditions are as reported previously [10,23]. Data shown are averages of three determinations from a representative experiment. This experiment was repeated three times using three different membrane preparations with similar results.

Radioligand	Concn. (nM)	Radoiligand bound (fmol per mg protein)	
		Song membranes	microsomal-light membranes
[^{3}H]Prazosin	4	1846 ±92	1400 ±62
[^{3}H]Epinephrine	75	189 ± 2.9	91.4± 4.9
[^{3}H]Yohimbine	10	68.6± 4.3	228.6± 5.7
[^{125}I]Iodocyanopindolol	0.2	6.8± 0.3	18.4± 0.6

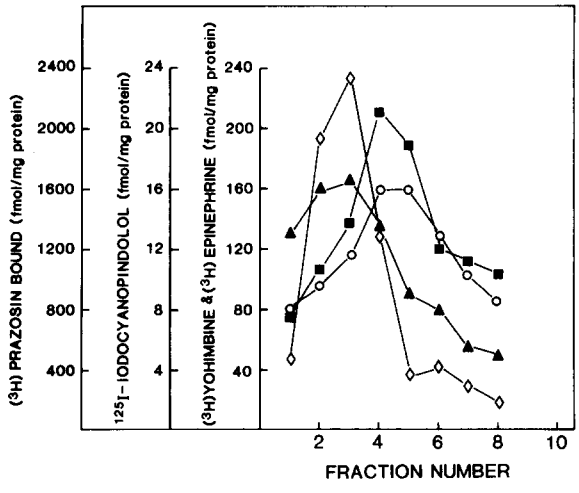

Fig. 1. Binding of adrenergic radioligands to sucrose gradient subfractions of Touster microsomal membranes. Ligands concentrations were: [^{3}H]prazosin, 4 nM (▲), [^{3}H]epinephrine, 75 nM (◇), [^{3}H]yohimbine, 10 nM (○), and [^{125}I]iodocyanopindolol, 0.2 nM (■). Protein concentrations in the subfractions were 2.5–0.3 mg/ml. Volumes of the subfractions used in the binding assays were 40, 150, 100, 60 μl for prazosin, epinephrine, yohimbine, and cyanopindolol, respectively. Data shown are from representative experiment repeated, with similar results, four times using three different preparations.

the microsomal-light fraction shown in Table I may be due to an enrichment of the yohimbine-binding site in those membranes.

Binding of the adrenergic radioligands to subfractions of the Touster preparation

To explore the possibility that the binding sites for various adrenergic radioligands detected in the microsomal-light fraction could be further separated, the Touster membranes were subfractionated by centrifugation on a linear sucrose gradient (8–40%, w/v) as explained in Methods. Fig. 1 shows the specific binding per mg protein of th various adrenergic radioligands to different subfractions. The ratios of specific binding to total binding were not significantly different in all fractions. To eliminate the influence of the differences in the constituents of the subfractions as a result of small experimental variations in the sucrose gradients, the binding of the various radioligands were carried out using the same subfraction from a single tube. It is seen that the peaks of specific binding (corrected for protein concentration) for prazosin and epinephrine are clearly separated from those for yohimbine and cyanopindolol. Sucrose concentrations up to 20% in the binding assay have no detectable effects on the binding of various adrenergic radioligands (data not shown). Since epinephrine has been shown to bind primarily to α_1-adrenergic binding sites in the liver [3,23,36], these data suggest that prazosin- and epinephrine-binding sites, which are of the α_1 subtype, may be physically separated from the binding sites for the α_2-adrenergic antagonist (yohimbine) and the β-adrenergic antagonist (cyanopindolol).

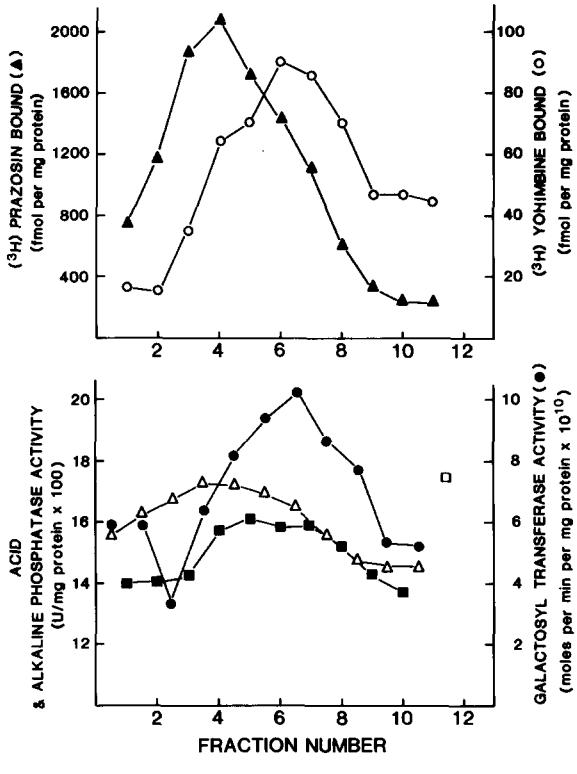

Fig. 2. Marker enzyme activity profiles in the linear sucrose gradient. The lower panel shows the activities of acid phosphatase (■), alkaline phosphatase (△), and galactosyl transferase (●) in the sucrose gradient subfractions. Acid phosphatase activity in the homogenate is indicated by the symbol [□]. The upper panel shows the binding of [^{3}H]prazosin and [^{3}H]yohimbine to the same subfractions for calibration purposes. Data are from representative experiment repeated three times with similar results.

Marker enzyme activities in the linear sucrose gradient subfractions

The lower panel in Fig. 2 shows the activities of the lysosomal marker enzyme, acid phosphatase

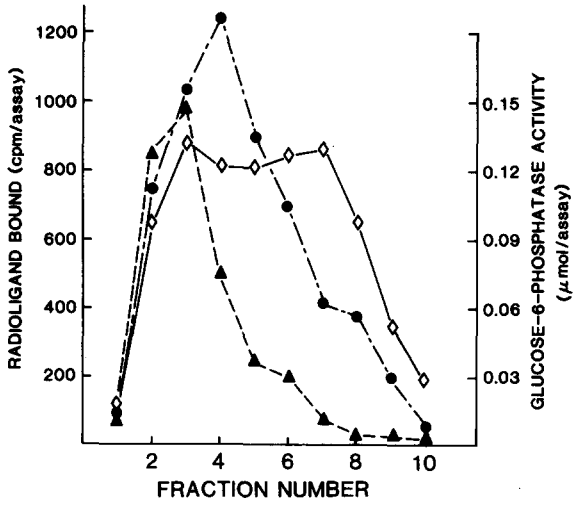

Fig. 3. Activity profile of glucose-6-phosphatase (◇) in the sucrose linear gradient. The activity of glucose-6-phosphatase was determined in the subfractions as explained in the text. The binding of [^{3}H]prazosin (▲) and [^{3}H]yohimbine (●), at 4 nM and 10 nM, respectively, to the same subfractions is included for calibration purposes. These data are from one representative experiment repeated three times with similar results.

[37,38], alkaline phosphatase, which is reportedly associated with plasma membranes [39], and the Golgi apparatus membrane marker enzyme, galactosyl transferase [30,31]. To calibrate the sucrose gradient, the results from binding experiments of [^{3}H]prazosin and [^{3}H]yohimbine to the same subfractions are shown in the upper panel. It is seen that both acid phosphatase and galactosyl transferase activities correlate well with the peak for [^{3}H]yohimbine binding (correlation coefficient > 0.96). However, there is no increase in the activity of acid phosphatase (corrected for protein) in those subfractions compared with that in the homogenate. This may indicate no enrichment of the lysosomal membranes in the subfractions. The activity peak for alkaline phosphatase is wide. This peak of activity could not be correlated exclusively with either radioligand binding peaks. Similarly, the activity of the primarily plasma membrane marker enzyme, 5′-nucleotidase, could not be correlated exclusively with either radioligand binding peaks (data not shown).

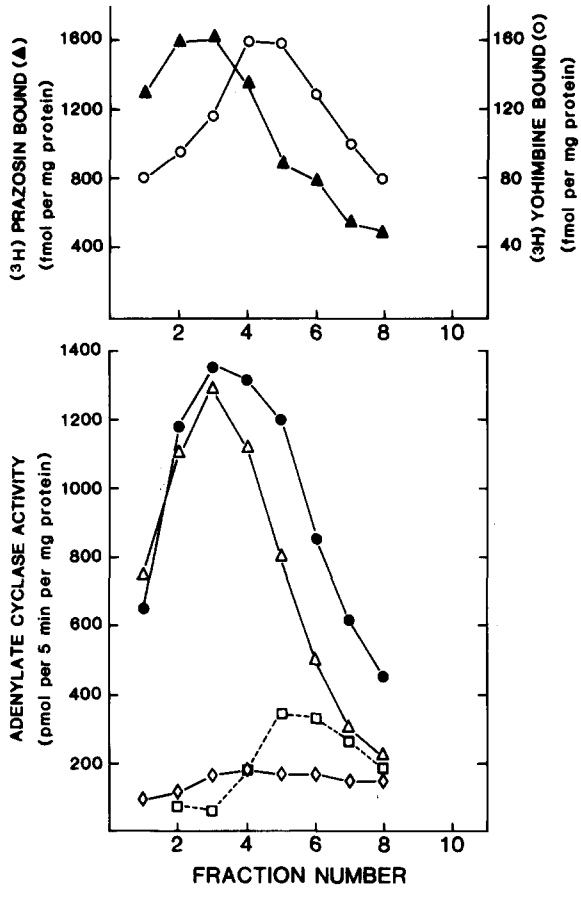

Fig. 4. Adenylate cyclase activity in the sucrose gradient subfractions. Lower panel, adenylate cyclase basal activity (◇) and stimulated activities in response to 0.1 μM glucagon (△) or 10 mM sodium fluoride (●) were determined as explained in the text. The differences in activity in the presence of glucagon and fluoride is also shown (□). The binding of 4 nM prazosin (▲) and 10 nM yohimbine (○) to the same subfractions is shown in the upper panel. Data are from one of two similar experiments.

Fig. 3 shows the distribution of the microsomal marker enzyme, glucose-6-phosphatase [38], in the linear sucrose gradient subfractions. It is seen that there is no clear correlation bteween the glucose-6-phosphatase activity and any of the binding peaks for [^{3}H]prazosin or for [^{3}H]yohimbine.

Fig. 4 (lower panel), shows the basal activity of the plasma membrane enzyme adenylate cyclase as well as its response to glucagon and sodium fluoride in the linear sucrose gradient subfractions. It is seen that the peak of the glucagon-activatable adenylate cyclase activity correlates well with the binding peak for [^{3}H]prazosin (Fig. 4, upper panel). It is also observed that the distribution of the fluoride-activatable adenylate cyclase is different from that of the glucagon-activatable activity. Subtraction of these two activities (glucagon and fluoride stimulated) revealed a fluoride-activatable peak which correlate with [^{3}H]yohimbine binding peak (Fig. 4, upper panel).

These results are consistent with the notion, that plasma membranes are present in most of the linear sucrose gradient subfractions containing measurable protein; and, that [^{3}H]yohimbine binding peak may indicate the presence of binding sites for this radioligand either in the Golgi membranes or in portions of the plasma membranes which may co-sediment with the Golgi membranes.

Effects of pretreatment of rat liver with concanavalin A

Scarborough [40] has introduced a method to isolate plasma membranes from cultured Neurospora crassa using concanavalin A. The reported basis for the usefulness of this method involves a cross-linking reaction that stabilizes the plasma membranes and prevents fragmentation and vesiculation during the homogenization and isolation processes [19,41]. Treatment of cells with con-

canavalin A results in the formation of plasma membrane fragments that migrate as more uniform particles to heavier densities on sucrose density gradients upon centrifugation. Harden et al. [42] have used a similar technique to isolate plasma membranes from astrocytoma cells. They have shown that in the presence of concanavalin A β-adrenergic receptors and adenylate cyclase activity migrate predominantly as a single sharp band at higher sucrose density than that seen without the use of concanavalin A [42,43]. We have utilized this technique in an effort to further identify the source of the membrane fraction rich in [^{3}H]yohimbine binding sites. Rat livers were perfused with a perfusion medium [25] containing 0.5 mg/ml convanavalin A, for 20 min. The livers were then perfused with 50 ml of medium containing no concanavalin A in order to wash out excess free concanavalin A. Membranes fractions were then prepared as explained in Methods from these concanavalin A-pretreated livers.

Fig. 5 shows the binding of the radioligands [^{3}H]prazosin, [^{3}H]yohimbine, and [^{125}I]-iodocyanopindolol to linear sucrose gradient subfractions of membranes from concanavalin A-treated rat livers. A definit shift to the left of the binding peaks for yohimbine and cyanopindolol can be observed. A shift of the [^{3}H]prazosin binding peak to the left is also detected. It is further

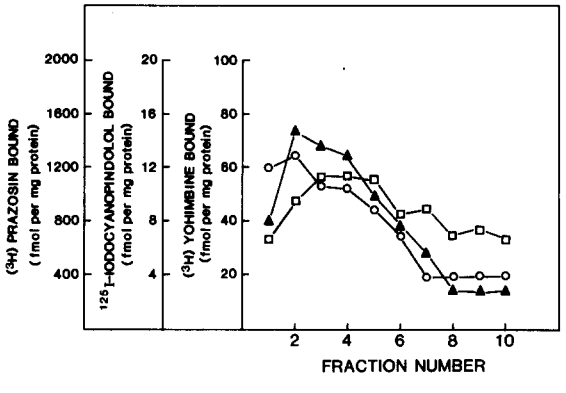

Fig. 5. Adrenergic radioligand binding to sucrose gradient subfractions from concanavalin A pretreated livers. Binding of 4 nM [^{3}H]prazosin (▲), 10 nM [^{3}H]yohimbine (○), and 0.2 nM [^{125}I]iodocyanopindolol (□) was carried out as explained in the text and in the legend to Fig. 3. The data are from representative experiment repeated, with similar results, three times using membranes from three different membrane subfraction preparations.

seen that there is no distinct separation of the binding peaks for yohimbine, prazosin and cyanopindolol in these membrane subfractions. Fig. 6, shows the activities of the various marker enzymes (lower panel). For calibration purposes, the upper panel shows the binding activities for yohimbine and prazosin to the same subfractions. The activity of alkaline phosphatase is shown to migrate in a sharper and distinct peak as compared to the peak of activity observed in the case of untreated livers (Fig. 2). Concanavalin A treatment also caused a noticeable shift to the left of the alkaline phosphatase activity peak. On the other hand, there is no significant changes in the activity distribution of acid phosphatase or galactosyl transferase. Fig. 7 shows the adenylate cyclase activities in response to glucagon and fluoride in membrane subfractions from concanavalin

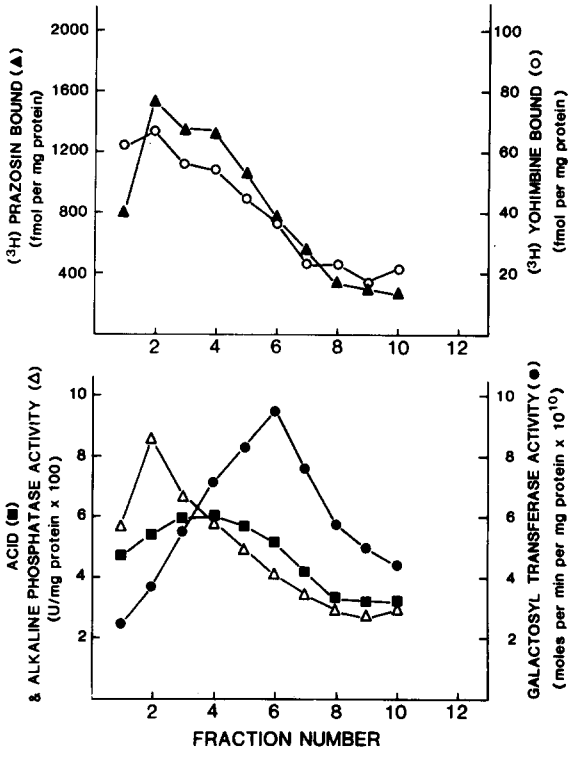

Fig. 6. Activities of marker enzymes in the subfractions of membranes from concanavalin A-treated livers. Enzyme activities were determined as explained in text and in the legend to Fig. 2. Results shown are from a representative experiment repeated, with similar results, three times using three different membrane preparations.

A-treated livers. A shift to the left of the glucagon-stimulated adenylate cyclase activity which corresponds with the prazosin binding peak is observed. It is also seen that the peaks of glucagon- and fluoride-stimulated adenylate cyclase activities coincide. This indicates a greater shift to the left of the fluoride mediated activity as a result of pretreatment of livers with concanavalin A.

Although a general decrease in the specific binding of the radioligands to the subfractions from concanavalin A treated livers was observed, binding experiments in the presence or absence of concanavalin A showed no direct effects of concanavalin A on the binding of prazosin, yohimbine, epinephrine, or cyanopindolol. In addition, no significant difference in radioligand binding to homogenates from concanavalin A-treated and untreated livers was observed. The lack of direct effect of concanavalin A indicated that the observed reduction in the binding of the radioligands may be due to a decrease in the plasma membranes content of the Touster preparation as a result of liver pretreatment with concanavalin A. The activities of the enzyme markers support this conclusion. Data presented in Figs. 2 and 6 show that there is no significant change in the total activity of the galactosyl transferase while a significant decrease (50–60%) in the total activity of alkaline phosphatase can be observed. These results indicate a reduction of the plasma membrane fraction in the microsomal preparation caused by pretreatment with concanavalin A.

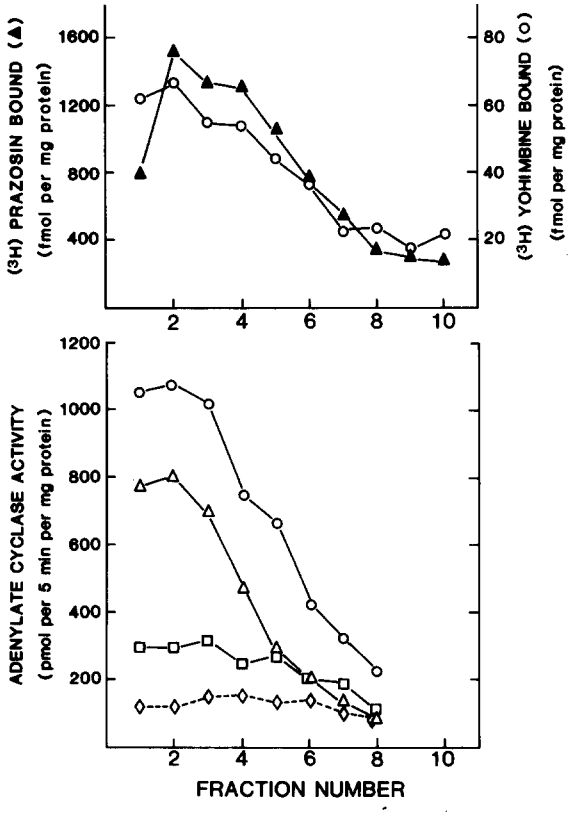

Fig. 7. Adenylate cyclase activity in membrane subfractions from concanavalin A-pretreated livers. The profile of basal activity (◇) and the stimulated activity using 0.1 μM glucagon (△), or 10 mM sodium fluoride (○) in the sucrose gradient subfractions are shown in the lower panel. The difference in activity in the presence of glucagon and fluoride is also shown (□). The upper panel shows the binding of prazosin and yohimbine to the same subfractions. Data are from experiment which was repeated once.

Discussion

Using plasma membrane fractions prepared according to the method of Song et al. [20] or Touster et al. [21] for the binding of α_1-, α_2-, and β-adrenergic radioligands, we have shown a heterogeneous distribution of binding sites for these ligands. Specific binding per mg protein for prazosin and epinephrine, which bind primarily to α_1-adrenergic binding sites in the rat liver [3,4,23,36,44], is shown to be greater in the Song membranes as compared to their binding in the Touster membranes. In contrast, specific binding per mg protein for the α_2- and β-adrenergic binding sites is lower in the Song membranes than in the Touster membranes. Further fractionation of the microsomal membranes on linear sucrose gradient revealed different profile for the binding of each of the two groups of radioligands to the sucrose gradient subfractions. The peaks of binding activity for prazosin and epinephrine are clearly separated from those for yohimbine and cyanopindolol which appear at lower sucrose density. Marker enzyme activities in the sucrose gradient subfractions, in agreement with published reports [17–19,22,37,38,41], indicate the presence of plasma membranes in almost all the sucrose gradient subfractions containing measurable protein. The binding peaks for the adrenergic radioligands cannot be correlated with the presence of mi-

crosomes or lysosomes. However, the possibility of yohimbine and cyanopindolol binding to Golgi membranes could not be ruled out using membrane subfractions obtained by established isolation methods. The presence of a significant contamination by mitochondria is believed to be unlikely [21,22,37].

In order to investigate the possibility of yohimbine and cyanopindolol binding to Golgi membranes, less fragmented plasma membranes were prepared from concanavalin A-pretreated livers. Scarborough [40] was the first to use concanavalin A pretreatment to prepare relatively pure plasma membranes from *Neurospora crassa.* It is believed that one of the major problems in the isolation of relatively pure plasma membranes is the fragmentation and vesiculation which occur upon the breaking of the cells. This leads to vesicles of nonuniform density and extensive smearing in isopycnic centrifugation procedures. Concanavalin A supposedly prevents fragmentation and vesiculation by a cross-linking reaction that stabilizes the plasma membranes. Our use of livers pretreated with concanavalin A in plasma membrane preparation resulted in a slight to the left shift of the binding of prazosin. More importantly the binding peaks for yohimbine and cyanopindolol are now shifted to sucrose gradient subfractions of higher density resulting in no distinct separation of the binding peaks of prazosin, yohimbine and cyanopindolol. The galactosyl transferase activity is shown to remain in relatively lighter density subfractions in a position comparable to that seen without concanavalin A pretreatment. This series of experiments clearly rules out the possibility that the binding of yohimbine and cyanopindolol is to membranes derived from the Golgi apparatus. This is in agreement with the reported observation that concanavalin A does not penetrate the cell and therefore remains associated only with the cell surface membranes [40]. Consistent with this fact is the distinctive left-shift of the activity peaks of alkaline phosphatase and adenylate cyclase in the sucrose gradient subfractions from concanavalin A-pretreated livers. Based on these observations we propose the hypothesis that yohimbine and cyanopindolol are present in areas of the plasma membranes which are different from the areas where the binding sites for prazosin and epinephrine are concentrated.

Plasma membrane fractions obtained by various methods originate primarily from each of the three major functional domains of the hepatocytes surface [17,22]. Membranes obtained by the method of Song et al. [20] are enriched in the canalicular region [19,20,41]. However parts of the mostly nonvesicular contiguous face are also included [19,22]. Although most hormone receptors should be located at the blood-sinusoidal face of hepatocytes in order for them to be accessible to circulating hormones, it has been reported that the contiguous face may be accessible due to diffusion into the interhepatic space [22,45]. In addition to that, neurotransmitters which are released from nerve ending (e.g. norepinephrine) may have access to any area of the plasma membranes. The microsomal fraction prepared according to Touster and his coworkers consists primarily of the sinusoidal face of the hepatocytes [21,22]. According to these reports as well as the results obtained in the present paper, the α_1-adrenergic binding sites and possibly the glucagon receptors [21,22] may be concentrated in the contiguous face whereas the α_2- and the β-adrenergic binding sites are located primarily on the blood-sinusoidal face of the hepatocytes. These results also raise the possibility that the receptors for glucagon and β-adrenergic agonists are associated with two different adenylate cyclase enzymes. The physiology of the hepatic α_1-adrenergic system indirectly supports this concept. This is because of the relatively high concentration of catecholamines required for activating half maximally the enzyme glycogen phosphorylase (K_{50} is 50 nM [23]). Such high concentrations of catecholamines are not likely to be present in the blood. Exton and coworkers have proposed that the α_1-adrenergic system may operate through the sympathetic nervous system [46].

This proposed architectural organization of the α_1- and β-adrenergic binding sites is also supported by the reported differences in the properties of the two systems. The rapid and large changes in the number of β-adrenergic binding sites due to alteration in the state of the animals (fasting [10], adrenalectomy [8], thyroidectomy [9]) may be explained by the presence of these binding sites on the sinusoidal side of the hepatocytes. Also the susceptibility of the β-adrenergic and not the α_1-

adrenergic receptors to desensitization [13–16] can be explained in a similar fahsion. These findings may be useful in lending insight into the turnover process for these binding sites.

Acknowledgements

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