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SOLUBILIZATION OF LIGAND-STABILIZED VASOPRESSIN RECEPTORS FROM PLASMA MEMBRANES OF BOVINE KIDNEY AND RAT LIVER

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SUMMARY: The solubilization of vasopressin receptors from plasma membranes of bovine kidney and rat liver by different detergents was investigated. A prerequisite for the extraction of vasopressin receptors retaining binding affinity for their ligand was the stabilization of the receptors by the prior formation of the membrane-bound hormone-receptor complexes. The vasopressin-receptor complexes from both kidney and liver membranes were solubilized in a high yield with dodecyl-ß-D-maltoside and 3-laurylamido-N,N'-dimethylpropylaminoxide. Several other nonionic detergents including octyl-ß-D-glucopyranoside effectively extracted the hepatic vasopressin receptor. For the hormone-receptor complex solubilized from bovine kidney with dodecyl-ß-D-maltoside, a Stokes' radius of 5.8 nm was determined.

The neurohypophyseal peptide hormone vasopressin interacts with different classes of receptors in plasma membranes: Its hormonal effects in the kidney are mediated by increased formation of cyclic AMP via stimulation of membrane-bound adenylate cyclase (1). Interaction of vasopressin with its receptor in rat liver membranes leads to Ca^{2+}-mediated glycogenolysis (2,3). Progress in the purification of vasopressin receptors and in the reconstitution of a vasopressin-sensitive system has been limited by the sensitivity of this hormone receptor to mild solubilization procedures. Experiments to extract membrane-bound vasopressin receptors have been performed until now only with the nonionic detergent Triton X-100 (4,5). We, therefore, performed a systematic study concerning the solubilization of vasopressin receptors from bovine kidney and rat liver by polyethoxy-type nonionic detergents, medium chain ß-1-alkylglycosides, zwitterionic

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detergents and bile salts. Furthermore, the stability and Stokes' radii of the hormone-receptor complexes in solution were determined.

MATERIALS AND METHODS

Materials. [(3.5-^{3}H)Tyr^2][Arg^8]vasopressin ([^{3}H]vasopressin) with a specific radioactivity of 6.8 Ci/mmol was prepared and purified as described previously (6). [8-Arginine]vasopressin was obtained from Bachem. Aminoxide[2] was a gift from Goldschmidt AG, Essen. Other detergents were purchased from chemical companies. Ultrogel AcA 34 was obtained from LKB, Sephadex G-50 from Pharmacia Fine Chemicals and [1-^{14}C] oleic acid from Amersham Buchler.

Preparation of membrane-bound vasopressin receptors. Plasma membranes from bovine kidney inner medulla containing high affinity binding sites for vasopressin were isolated and binding affinity and capacity were measured as in (6).

Liver plasma membranes with a binding capacity of 2.6 pmol [^{3}H]vasopressin/mg membrane protein were prepared by using an aqueous two phase polymer system (7). Protein was determined by the Lowry method with modifications as described in (8) and (9).

Solubilization of vasopressin-receptor complexes. Membranes were incubated for 20 min at 30° C with 10^{-8}M[^{3}H]vasopressin in binding medium containing 50 mM Hepps buffer (pH 8.3 for renal membranes, pH 7.8 for hepatic membranes) and 3 mM $MgCl_2$. After cooling to 0° C, free [^{3}H]vasopressin was removed by pelleting the membranes and resuspending them in binding medium. Detergent extracts (total volume 200 µl) were prepared by incubating the membrane suspensions (4 mg protein/ml) at 0° C for 30 min with an equal volume of 200 mM Hepps buffer (pH 8.3 for renal membranes, pH 7.6 for hepatic membranes) containing 10 mM $MgCl_2$ and different concentrations of detergent, NaCl and glycerol. The mixtures were sedimented at 200 000xg for 60 min and the supernatants (75 µl) were filtrated over Sephadex G-50 columns (0.5x7.5 cm) to separate bound and free hormone. Radioactivity eluting in the void volumn V_0 of the columns (V_0=0.5-1.7 ml) represents the total binding of [^{3}H]vasopressin and was measured by counting the eluate

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[2] Abbreviations used: Aminoxide, Aminoxide WS 35, mixture of 3-laurylamido-N,N'-dimethylpropylaminoxide (55 %) and longer chain aminoxides (C_{14}, C_{16}, C_{18}); Brij 96, polyoxyethylene (n=10) oleyl ether; $C_{12}E_9$, polyoxyethylene (n=9) lauryl ether; dodecyl maltoside, 1-O-n-dodecyl-ß-D-maltoside; Hepps, 4-(2-hydroxyethyl)-1-piperazinepropanesulphonic acid; Lubrol PX, polyoxyethylene (n=9.5) lauryl and myristyl ether; octyl glucoside, 1-O-n-octyl-ß-D-glucopyranoside.

with 10 ml Aqualuma Plus (LKB). It was corrected for non-specific binding which was determined by incubating either the membranes with an excess ($4x10^{-6}$ M) of unlabelled vasopressin or the detergent extract with an equal volume of 1 M ammonium acetate buffer pH 5. Both methods eliminate the specific binding of [^{3}H]vasopressin.

RESULTS

Vasopressin-receptor complexes were formed by incubating plasma membranes from bovine kidney and rat liver with 10^{-8} M [^{3}H]vasopressin. At this concentration the radioactive ligand occupied more than 90 % of its specific binding sites in the membranes; nonspecific binding to membranes and to the detergent extracts represented less than 10 % of the total binding.

The vasopressin receptor from rat liver was effectively solubilized as hormone-receptor complex by several nonionic detergents (Fig. 1). The conditions were optimized with regard to the concentrations of detergent and electrolytes and the effect of glycerol (Tab. 1): Glycerol remarkably increased the yields obtained with the bile salt, sodium-taurodeoxycholate, and with octyl glucoside. Increasing the ionic strength by NaCl led to a drastic decrease in the efficiency of these two detergents (while having minor effects on others). Yields of solubilization below 5 % were obtained

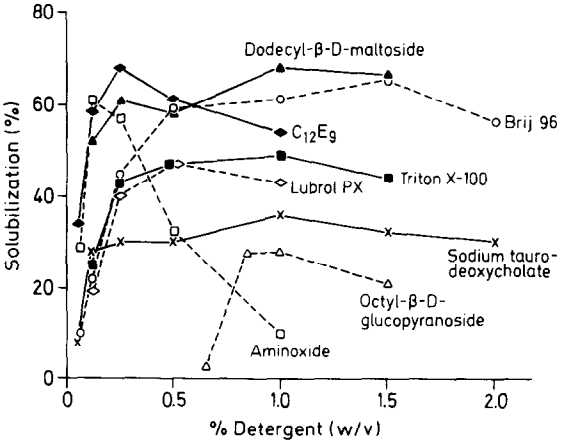

Figure 1. Solubilization of the vasopressin receptor complex from rat liver. Results are the percentage of [^{3}H]vasopressin receptor complexes which can be extracted from the plasma membrane by treatment with different concentrations of detergent.

Table 1. Optimal conditions for solubilization of the vasopressin receptor complex R-H from rat liver membranes

Detergent	Optimal concentrations of detergent	Optimal concentrations of sodium chloride	Glycerol 10 %, (v/v)	Solubilization of R-H	Solubilization of protein
	% (w/v)	mM		%	
Dodecyl maltoside	1-1.5	50	-	74	51
Brij 96	1-1.5	100	+	65	34
$C_{12}E_9$	0.25	0	+	64	41
Triton X-100	0.5-1.5	200	+	62	44
Aminoxide	0.15	50	-	61	36
Sodium taurodeoxycholate	0.75-1.5	0	+	51	42
Lubrol PX	0.5	0	-	49	35
Octyl-glucoside	1	0	+	42	33

by polyethoxy type nonionic detergents (Brij 56, Lubrol WX and Tween 80), by zwitterionic detergents (N-alkyl sulfobetaines and lysolecithin) and by the bile salts sodium cholate and deoxycholate.

The vasopressin receptor from bovine kidney displayed a different behaviour. Only dodecyl maltoside and aminoxide solubilized more than 25 % of the membrane-bound hormone-receptor complex. At the optimal detergent concentration of 0.25 % (w/v), dodecyl maltoside solubilized 82 % of the renal hormone-receptor complex (Fig. 2) and 41 % of the total membrane proteins. By 0.3 % aminoxide 65 % of the hormone-receptor complex and 37 % of the proteins were extracted from the plasma membrane of bovine kidney inner medulla. Addition of glycerol (10 %; v/v) to aminoxide increased the yield of the soluble renal hormone-receptor complex to about 90 %. The hormone-receptor complexes were more labile in micellar solutions of non-ionic detergents than the membrane-bound complexes (Fig. 3): The apparent dissociation of the renal vasopressin-receptor complex in detergent solution at 2^{o} C was biphasic with a high rate of dissociation during the first 10 h, followed by a slower decay. The time course of dissociation indicates a nearly 100 % extraction of the membrane-bound hormone-receptor

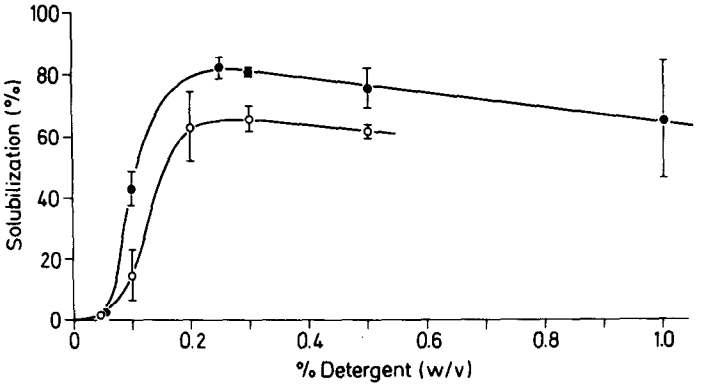

Figure 2. Solubilization of the vasopressin receptor from plasma membranes of bovine kidney inner medulla by treatment with dodecyl maltoside (●) and aminoxide (○).

complex which partially dissociates during the separation of membrane extract and residual pellet.

When the optimal conditions for the solubilization of hormone-receptor complexes from rat liver and bovine kidney membranes were applied to extract the receptors in the non-liganded state, the binding capacity in the residual membrane pellet was greatly reduced. No specific binding of

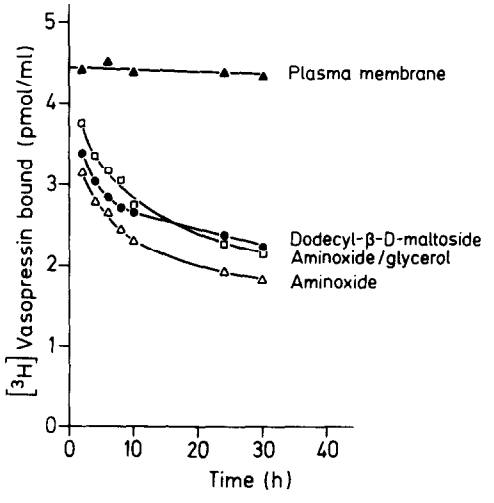

Figure 3. Apparent dissociation of membrane-bound and solubilized vasopressin receptor complexes from bovine kidney. Aliquots of the membrane-bound hormone-receptor complex were solubilized with 0.3 % dodecyl maltoside (●), 0.3 % aminoxide (△) and 0.3 % aminoxide/10 % glycerol (□). The amonut of hormone-receptor complexes remaining at 2^{0} C after different times was determined as described in "Materials and Methods".

[^{3}H]vasopressin in the detergent extract was found.

The Stokes' radii of the vasopressin-receptor complexes in detergent solution were determined by gel filtration. Fig. 4 shows the elution profile of the soluble extract obtained with dodecyl maltoside from membrane of bovine kidney. The first peak of radioactivity corresponded to [^{3}H]vasopressin bound to its receptor, the second peak to [^{3}H]vasopressin which has dissociated from the solubilized receptor during gel filtration. The elution volume of detergent micelles was indicated by the position of [1-^{14}C]oleic acid which incorporates into these micelles (10). The specificity of [^{3}H]vasopressin binding to its solubilized receptor was demonstrated by experiments where an excess of unlabelled vasopressin was added to the membranes either before or after solubilization. In both cases

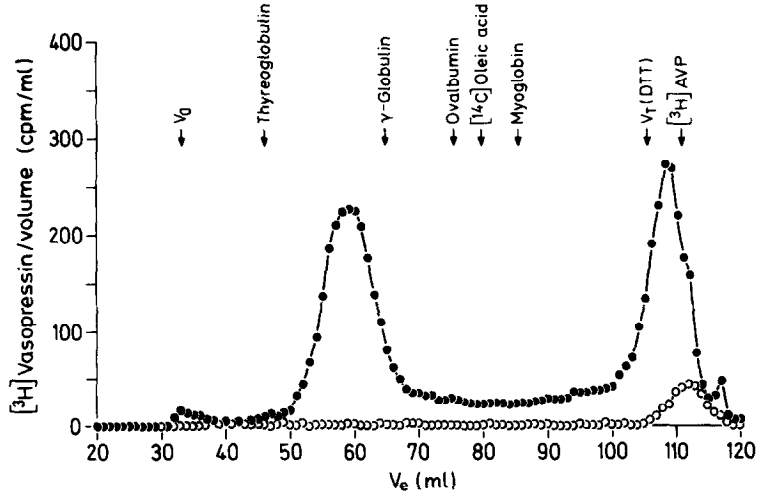

Figure 4. Gel filtration of the vasopressin receptor complex from bovine kidney inner medulla. A membrane preparation solubilized in dodecyl maltoside (200 µl) was chromatographed on an Ultrogel ACA 34 column (1.6x50 cm) at a constant flow rate of 6 ml/h. The column was equilibrated at 4° C with 0.1 % dodecyl maltoside, 50 mM Tris HCl pH 8.5, 50 mM NaCl and 5 mM $MgCl_2$. Fractions of 1 ml were counted with 4 ml Aqualuma plus (●). In a control experiment plasma membranes were incubated in addition to [^{3}H]vasopressin with an 100-fold excess of unlabelled vasopressin (○). Arrows indicate the elution positions of marker proteins, [1-^{14}C]oleic acid and [^{3}H]vasopressin. V_t and V_o represent the total and void volumes of the column, V_e the elution volumes.

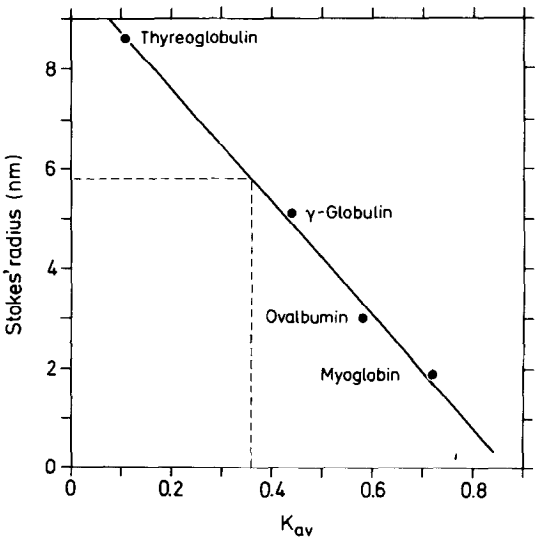

Figure 5. Estimation of the Stokes' radius of the vasopressin receptor complex from bovine kidney. The curve was constructed by plotting the known Stokes' radii of marker proteins against their partition coefficients $K_{av}(K_{av}=V_e-V_o/V_t-V_o)$. Values for V_e, V_t and V_o are from Fig. 4; the values for the Stokes' radii of globuline, ovalbumin and myoglobin have been taken from Tanford et al. (13), for thyroglobulin from Le Maire et al. (14).

only the second peak of radioactivity corresponding to free [^{3}H]vasopressin was found. From the plot of partition coefficients K_{av} for the hormone-receptor complex and for marker proteins against Stokes' radii (Fig. 5), a Stokes' radius of 5.8 nm was determined for the vasopressin receptor-detergent complex from bovine kidney. This value is very similar to the values of 5.6 and 5.4 nm for Triton X-100 solubilized hormone-receptor complexes from rat kidney and rat liver, respectively (5).

DISCUSSION

The change in environment of the vasopressin receptor from the native lipid bilayer to that of the detergent-lipid mixed micelle results in a decrease in stability: the rate of dissociation of the soluble hormone-receptor complexes is greater than that of the membrane-bound complexes. Solubilization of vasopressin receptors in the non-liganded state leads to a loss of their hormone-recognition.

The results of the present study render reconstitution experiments with solubilized vasopressin receptors possible. Such experiments should show whether the incorporation of vasopressin receptors into liposomes can preserve their binding affinity and their functional interaction with other membrane proteins involved in the signal-transfer. For the nonionic detergents dodecyl maltoside and aminoxide, which extract the hormone-receptor complexes almost completely from renal and hepatic membranes, procedures have been described (11,12) to incorporate solubilized proteins into liposomes and to reconstitute functional systems. The high critical micelle concentration of octyl glucoside should allow the incorporation of the hepatic vasopressin receptor into liposomes by rapid detergent dilution or dialysis.

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