Effect of various solubilizers on angiotensin$_{II}$ receptors in bovine adrenocortical plasma membranes[1]

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Summary. 3 nonionic detergents, Triton X-100, Lubrol WX and NP-40, inhibited binding of [^{3}H]-AT$_{II}$ to bovine adrenocortical plasma membranes. This effect appeared to be direct and not due to solubilization of the AT$_{II}$ receptor by these agents. Sodium deoxycholate and the chaotropic ions, ClO_4^- and Br^-, produced effects similar to the nonionic detergents.

Several studies relating to plasma membrane receptors for angiotensin$_{II}$ (AT$_{II}$) have been reported in particulate fractions derived from aorta and adrenocortical glands of different species[2-6]. The accuracy of AT$_{II}$ binding kinetic studies and characterization of receptor material in these studies, is undoubtedly influenced to some extent by such factors as high, nonspecific binding of the ligand, presence of catabolic enzyme activity and heterogeneity of membranes. To minimize these problems AT$_{II}$-receptor interactions could best be studied using purified receptor preparations. Devynck et al.[7] have had some success in solubilizing the rabbit aortic AT$_{II}$ receptor; the current study was undertaken to isolate the AT$_{II}$ receptor from bovine adrenocortical plasma membranes.

Methods and materials. Bovine adrenocortical membranes were prepared according to the previous techniques of Forget and Heisler[5]. Membranes were preincubated for 15 min (22 °C) in 100 mM Tris-HCl (pH 7.5) containing 120 mM NaCl and 7.2×10^{-8} M [^{3}H]-AT$_{II}$. Binding equilibrium at 22 °C is achieved within 10 min and is stable thereafter to 30 min[5]. Control tubes contained an additional amount of nonisotopic AT$_{II}$ (5×10^{-5} M). Following preincubation, surfactants or chaotropic ions and additional [^{3}H]-AT$_{II}$ (7.2×10^{-8} M) were added and mixtures further incubated for 10–60 min (22 °C). Surfactants or chaotropic ions were not added to control tubes.

One of 2 of the following procedures was employed at the end of the incubation period: a) sample mixtures were subjected to millipore filtration (0.45 µm) and specific [^{3}H]-AT$_{II}$ binding to the remaining, nonsolubilized fraction (residue), quantitated as a percentage of control binding; b) sample mixtures were centrifuged at 105,000 × g for 60 min. Protein content in the supernatant was determined by the method of Lowry et al.[8] or the modified method of Dulley and Grieve[9]. Aliquots of supernatants were subjected to gel chromatography on biogel P4 (Bio-Rad, Richmond, Ca). Columns (0.7 × 50 cm) were equilibrated in 100 mM Tris-HCl (pH 7.5) containing 120 mM NaCl and were washed with 2 bed volumes of the same buffer containing either surfactant or chaotropic ion, prior to use. Columns were eluted in the same buffer and radioactivity in 0.5-ml fractions was quantitated.

Alternatively aliquots of the 105,000 × g supernatant were applied to Whatman No 1 chromatography paper and developed (ascending) in n-butanol-acetic acid-water (25:4:10 v/v) for 18 h. Chromatograms were sectioned at 1-cm intervals and counted in Bray's solution. The technique is identical to the radiochemical purity assay described by the producer of the isotope.

AT$_{II}$ was obtained from Beckman Bioproducts (Palo Alto, Ca). [Tyrosyl-3, 5-^{3}H-(N)]-AT$_{II}$ was purchased from New England Nuclear (Boston, Ma). Surfactants were obtained from Sigma Chemicals (St-Louis, Mo). Nonidet P-40 (NP-40) is a product of Shell Chemicals. Chaotropic agents and other reagent grade chemicals were purchased from Fisher Scientific (Montreal, Que). Results are the means of at least 4 observations.

Results. The effects of 3 nonionic detergents on the [^{3}H]-AT$_{II}$ binding process are described in figure 1. All 3 detergents used – Triton X-100 (30 min incubation), Lubrol WX (30 min incubation) and NP-40 (10 min incubation) – reduced binding of the radioligand when compared to the binding process in nontreated membranes. Triton X-100 and Lubrol WX were equi-effective on a weight basis, both reducing [^{3}H]-AT$_{II}$ binding by 50% in a detergent: protein ratio of 1:18.5. NP-40 could also inhibit the binding process though relatively higher concentrations were required.

In order to ascertain whether the apparent inhibition in [^{3}H]-AT$_{II}$ binding was due to solubilization by the detergents of the membranes, samples underwent gel chromatography as described in 'methods'. Membranes were preincubated with 0.032% Triton X-100 or Lubrol WX (60 min) or 0.025% NP-40 (10 min) prior to centrifugation. The table indicates that each of the detergents did in fact solubilize, to a varying extent, membrane protein. Figure 2 illustrates the elution profile obtained with Triton X-100 following chromatography on biogel P4; results with the other detergents were qualitatively identical. No radioactivity was recoverable in the void volume; only 1 radioactive peak (98% recovery of added counts) was eluted from the column under the current experimental conditions. Unhy-

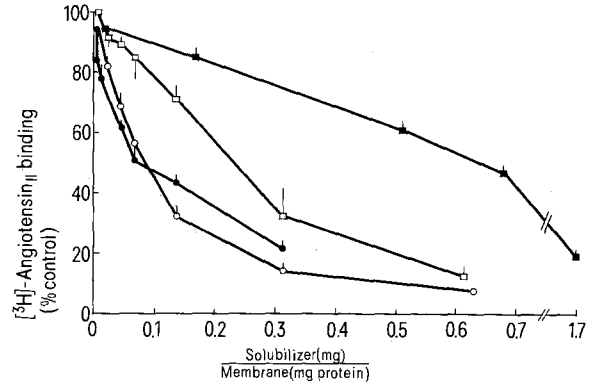

Fig. 1. Effect of various membrane solubilizers on [^{3}H]-AT$_{II}$ binding to adrenocrotical plasma membranes. Triton X-100 (●); Lubrol WX (○); sodium dexycholic acid (□); NP-40 (■). Control binding (pmoles AT$_{II}$ · mg protein^{-1} ± SE; n = 6) = 5.11 ± 0.28.

Solubilization of bovine adrenocortical plasma membranes by detergents and chaotropic ions

Agent	Concentration	Solubilization of initial amount of protein*
Triton X-100	0.032%	52.6 ± 4.1
Lubrol WX	0.032%	57.2 ± 5.3
NP-40	0.025%	32.1 ± 1.5
Sodium deoxycholate	0.032%	49.0 ± 3.6
$NaClO_4^-$	0.036 M	45.0 ± 1.5

* Values are means ± SE of 4–8 observations.

drolyzed [^{3}H]-AT_{II} is eluted with the same volume of buffer.

Radiochromatograms of [^{3}H]-AT_{II}, [^{3}H]-AT_{II} exposed to the various detergents (see table for concentrations), or of aliquots derived from the 105,000 × g supernatants following exposure of membranes to the various detergents, were identical. It is therefore evident that the reduction of [^{3}H]-AT_{II} binding observed in the preceding experiments is due to a direct effect of the detergents on AT_{II} recognition sites and is not related to solubilization of membrane enzymes capable of hydrolyzing the radioligand.

Sodium deoxycholate inhibited the [^{3}H]-AT_{II} binding process, as well (figure 1); however, as with the nonionic detergents this effect is apparently a direct one.

Chaotropic ions (ClO_4^-; Br^-) also inhibited the [^{3}H]-AT_{II} binding process (figure 3). In this respect the trichloroacetate ion was markedly more effective than the bromide ion. The trichloracetate ion, however was ineffective in solubilizing the AT_{II} receptor as were the previous detergents tested, though the ion did solubilize about 50% of membranal protein.

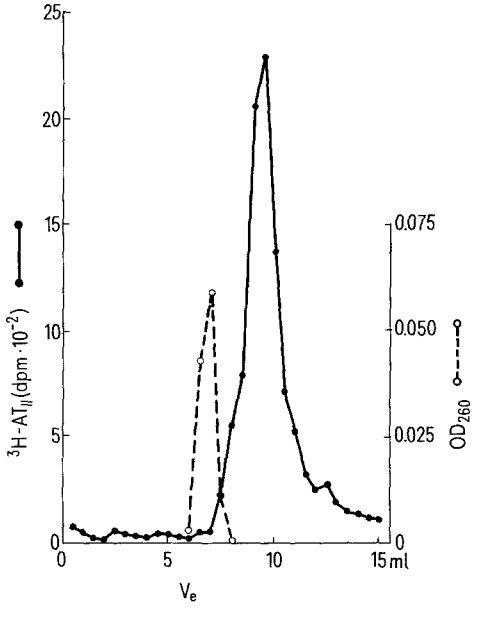

Fig. 2. Gel filtration pattern of a 105,000 × g supernatant of adrenocortical plasma membranes preincubated with [^{3}H]-AT_{II} and Triton X-100; prior to centrifugation. V_e = elution volume; OD of dextran blue (- - -).

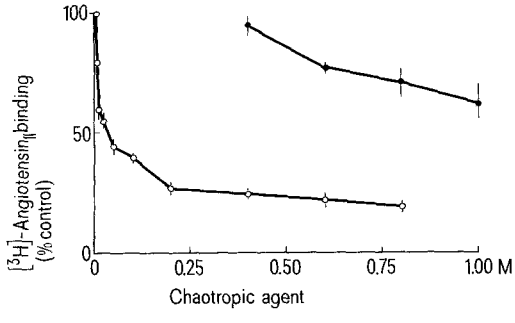

Fig. 3. Effect of chaotropic ions on [^{3}H]-AT_{II} binding to adrenocortical plasma membranes. $NaClO_4$ (○); NaBr (●). Control binding (pmoles AT_{II} · mg protein^{-1} ± SE; n = 3) = 3.74 ± 0.21.

Discussion. Nonionic detergents have been used to solubilize a number of peptide hormone receptors including those for insulin[10,11], glucagon[12], prolactin[13] and parathormone[14]. Protection of the hormone recognition site by prior application of ligand was not necessary in these studies; however, Catt and Dufau[15] found that unless this precaution was taken they were unable to solubilize the leutinizing hormone receptor.

Notwithstanding similar precautions in the current study, none of the nonionic detergents solubilized the protected AT_{II} receptor. In fact, AT_{II} binding capacity was reduced by them all. These findings suggest the possibility that membrane lipids may be essential constituents of the AT_{II} receptor structure. Recent studies in which phospholipases were demonstrated to inhibit the AT_{II} binding process (Brecher et al.[3]; unpublished data), support this suggestion. Devynck et al.[7] reported the solubilization of AT_{II} receptors in rabbit aortae using sodium dexycholate. These findings could not be duplicated in bovine adrenocortical plasma membranes which indicates that fundamental differences in receptor structure may exist between species and/or target organs.

In the past, chaotropic agents have also been used to solubilize membrane proteins. Recently, Tate et al.[16] used lithium diiodosalicylate to solubilize the thyrotropin receptor. Unlike nonionic detergents or biliary salts which act by delipidation, chaotropic ions have water structure-breaking characteristics which enable them to destabilize membranes, thereby increasing the solubility of resident nonelectrolytes[17]. However, neither of the chaotropic ions tested currently was capable of solubilizing the AT_{II} receptor.

The difficulty in solubilizing the adrenocortical AT_{II} receptor in these studies reflects both the complexity and differences between its structure and the nature of other peptide hormone receptors, in general.

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