IDENTIFICATION OF SPECIFIC VASOPRESSIN BINDING SITES ON CELL MEMBRANES OF RAT ADENOHYPOPHYSIS, LIVER, KIDNEYS, AND ADRENALS

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Receptors for vasopressin (VP), found in various peripheral tissues [3, 5] and (more recently) in some parts of the brain [1, 4] have aroused great interest because of the important role of this hormone in the maintenance of homeostasis and its putative role in a number of CNS functions [4, 9]. Two types of receptors for VP have been described in peripheral tissues: V_1 (pressor) receptors, regulating hydrolysis of inositol phosphates and intracellular Ca^{++} metabolism, found in particular in the walls of blood vessels, in platelets, the liver, and certain other organs; V_2 (antidiuretic) receptors, connected with adenylate cyclase and present, in particular, in the kidneys [6]. Evidence also has been obtained of the existence of VP receptors belonging to neither the V_1 nor the V_2 types [1, 5].

A method of detecting specific bindings sites of [^{3}H]-VP in the adenohypophysis, liver, and kidneys, and their properties are described in this paper, and the existence of specific binding sites for VP in the adrenals of rats also was established.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 180-200 g were used. The organs were removed quickly after decapitation, cooled on ice, and freed from connective tissue; the liver and kidneys were cut into pieces with scissors and the adenohypophysis was separated from the neurohypophysis. Weighed samples of tissue were homogenized in an "Ultraturrax" homogenizer ("Ika-Werke," West Germany) with No. 8 adapter for 30 sec at maximal speed in 20 volumes (liver and kidneys) or 100 volumes (adenohypophysis or adrenals) of cold (2°C) isolation buffer of the following composition: 50 mM Tris ("Serva"), 1 mM EGTA ("Sigma"), 1 mg/ml GABA, 50 μg/ml of bacitracin ("Sigma"), 20 U/ml of contrykal (VEB "Arzneimittelwerk," East Germany), 1 mg/ml bovine serum albumin ("Sigma"), acetic acid to pH 7.4. The resulting suspension was centrifuged for 20 min (20,000 g) at 4°C and the supernatant was discarded. Homogenization and centrifugation were repeated a further twice (liver and kidneys) or once (adenohypophysis or adrenals), after which the pellets were frozen at −20°C overnight. The frozen pellet was homogenized in isolation buffer and centrifuged under the same conditions, after which it was homogenized in cold (0°C) incubation buffer: 50 mM Tris ("Serva"), 1 mM EGTA ("Sigma"), 5 mM $MgCl_2$, 50 μg/ml bacitracin ("Sigma"), 1 mg/ml bovine serum albumin, acetic acid to pH 7.4 (20°C). The resulting suspension was used for determination of specific binding of [^{3}H]-VP. It must be pointed out that raising the temperature of the medium during preparation of the liver and kidney tissue to 10-15°C leads to a considerable decrease (by 20-60%) in specific binding. To determine specific binding of [^{3}H]-VP with kidney and liver cell membranes, the suspension was incubated in a volume of 1 ml of incubation buffer, with a tissue concentration of 10-40 mg/ml, calculated relative to the initial wet weight, in the presence of [^{3}H]-VP (^{3}H-argininevasopressin, 64 Ci/mmole, NET-800, "New England Nuclear") in "Eppendorf" polyethylene test tubes (1.8 ml) for 80 min at 4°C. In the case of cell membranes of the adenohypophysis and adrenals the volume of incubation mixture was 0.5 ml and the conditions of incubation were the same. After the end of incubation the samples were centrifuged for 10 min (2000g) at 4°C. The supernatant was poured off and the residue carefully washed with 1 ml of incubation buffer (4°C), after which the tubes were inverted on filter paper to remove the residual drops. The

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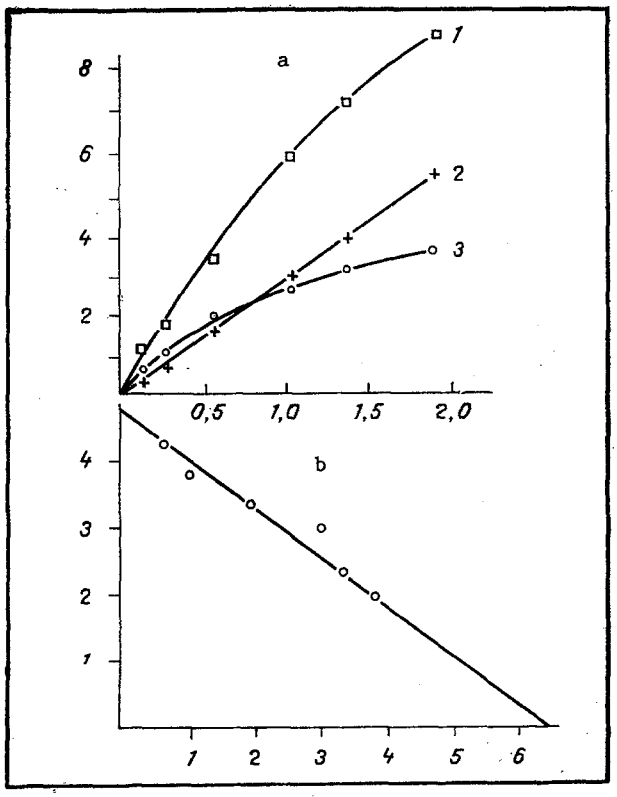

Fig. 1. Identification of specific binding sites of [^{3}H]-VP on rat liver cell membranes (data of one of six independent experiments; mean values given in text): a) equilibrium binding of [^{3}H]-VP with rat liver membranes. Membranes incubated for 80 min at 4°C with various concentrations (0.1-2 nM) of ligand. 1) Total binding; 2) nonspecific binding in presence of 2 μM argininevasopressin; 3) specific binding, difference between total and nonspecific binding. Abscissa: concentration of ligand (in nM); ordinate: binding (in pmoles/g tissue); b) Scatchard plot for specific binding of [^{3}H]-VP with rat liver membranes. Abscissa, specific binding (in pmoles/g tissue); ordinate, ratio of specific binding (pmoles/g tissue) to concentration of free ligand (nM). K_d 1.34 nM; B_{max} 6.53 pmoles/g tissue; r 0.89.

residues were suspended in 150 μl distilled water and transferred to scintillation vials, containing 10 ml of Bray's scintillator [2] for determination of radioactivity on a scintillation counter. Nonspecific binding was determined in the presence of 2 μM argininevasopressin ("Serva") and was subtracted from total binding to estimate specific binding. Total binding was determined in 2-4 and nonspecific in 2-3 parallel tests. To estimate the dissociation constant (K_d) and the concentration of binding sites (B_{max}), specific binding was determined in 5-6 concentrations of the ligand within the range from 0.1 to 4 nM, and the results were plotted between Scatchard coordinates, and a linear regression method was used.

EXPERIMENTAL RESULTS

Specific binding of [^{3}H]-VP with cell membranes of the liver, kidneys, and adenohypophysis was saturable and reversible. Specific binding increased with a fall of temperature of the incubation medium, both in absolute value and as a percentage of total binding. For instance, during incubation of liver cell membranes it was 10-15% at 35°C, 45-50% at 20°C, and 55-60% of total binding at 4°C, with a concentration of the ligand of 1 nM.

The presence of $MgCl_2$ in a concentration of 2.5 mM in the incubation medium increased specific binding of [^{3}H]-VP (1 nM) by 15-20%, and in concentrations of 5 and 10 mM, by

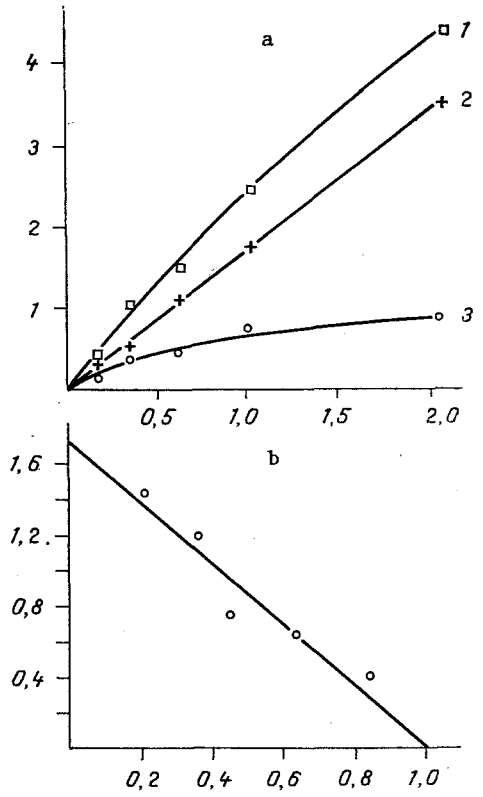

Fig. 2. Detection of specific binding sites for [^{3}H]-VP on rat kidney cell membranes (results of one of six independent experiments; mean data given in text). a) Equilibrium binding of [^{3}H]-VP with rat kidney membranes. Conditions of incubation and legend as in Fig. 1a; b) Scatchard plot for specific binding of [^{3}H]-VP with rat kidney membranes. Legend as to Fig. 1b. K_d 0.58 nM; B_{max} 1.02 pmole/g tissue; r 0.91.

25-30%. $NiCl_2$ also had a marked stimulating effect on binding of [^{3}H]-VP with cell membranes. For instance, when 5 mM $MgCl_2$ in the incubation medium was replaced by 5 mM $NiCl_2$ an increase in specific binding of the ligand with cell membranes of the adenohypophysis by 45% was observed. However, nonspecific binding of [^{3}H]-VP also was considerably increased on replacement of $MgCl_2$ by $NiCl_2$. For instance, for a concentration of the ligand of 1 nM an increase in nonspecific binding by 2.5-3 times was observed. Since the ratio of specific to total binding is higher than the presence of $MgCl_2$ (75%) then in the presence of $NiCl_2$ (55%), saturation analysis was carried out in incubation medium containing the magnesium salt. Under these conditions one class of binding sites for [^{3}H]-VP was found in liver tissue (Fig. 1): with K_d = 1.52 ± 0.50 nM and B_{max} = 11.8 ± 5.6 nmoles/g tissue (n = 6). The parameters of specific binding of [^{3}H]-VP in the kidneys were K_d = 0.66 ± 0.21 nM and B_{max} = 1.7 ± 0.48 pmole/g tissue, respectively (n = 6; Fig. 2). It will be clear that the concentration of specific binding sites in the liver tissue of rats is 6 times higher than their concentration in kidney tissue, in good agreement with data in [7]. The presence of one class of binding sites with K_d = 0.84 ± 0.21 nM and B_{max} = 4.9 ± 1.2 pmole/g tissue also was found on cell membranes of the adenohypophysis (n = 3; Fig. 3). Thus the method of isolation of a membrane preparation and determination of specific binding, used in the present investigation, enables specific binding sites of [^{3}H]-VP with affinity in the nanomolar range to be identified on cell membranes of the liver, kidneys, and adenohypophysis. Attention also is drawn to the fact that the concentration of binding sites for [^{3}H]-VP in the adenohypophysis was as much as half of their concentration in the liver tissue, and much more than their concentration in the kidneys where, as is considered [7], the concentration of VP receptors is among the highest in the body.

The value of specific binding of [^{3}H]-VP with cell membranes of the adrenals was considerably less than that in the other tissues examined in this paper. For instance, with

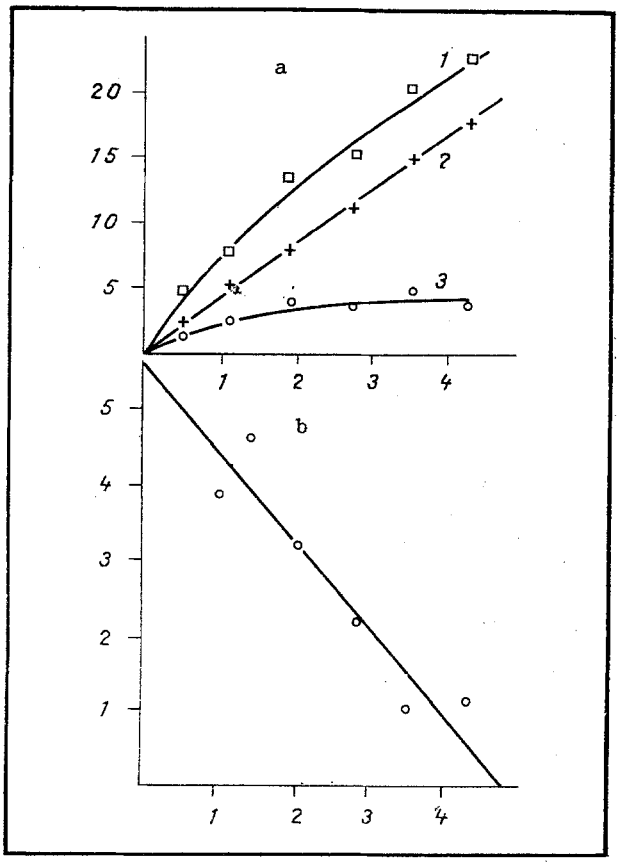

Fig. 3. Identification of specific binding sites of [^{3}H]-VP on cell membranes of the rat adenohypophysis (results of one of three independent experiments, mean data given in text). a) Equilibrium binding of [^{3}H]-VP with membranes of rat adenohypophysis. Conditions of incubation and legend as for Fig. 1a; b) Scatchard plot for specific binding of [^{3}H]-VP with membranes of adenohypophysis. Legend as to Fig. 1b.

a concentration of the ligand of 0.3 nM, specific binding of [^{3}H]-VP with adrenal cell membranes was 0.21 ± 0.09 pmole/g tissue, which is 6 to 8 times less than that observed in the adenohypophysis. Such a low concentration of binding sites does not allow saturation analysis to be carried out with the radioligand with specific activity of 64 Ci/mmole used in the present investigation. Specific binding of [^{3}H]-VP with adrenal cell membranes is of the saturation type and increases if $MgCl_2$ in the incubation medium is replaced by $NiCl_2$, evidence of its receptor nature. Data on the presence of vasopressin binding sites in the rat adrenals could not be found in the accessible literature. The ability of VP to potentiate growth of glomerular cells of the adrenals in culture and to induce release of aldosterone by the adrenals [7], observed previously, and the results of the present investigations together suggest that this hormone possesses yet another function, that of regulating secretory activity of the adrenal cortex. Activation by vasopressin of the release of catecholamines capable of inducing protein synthesis in the nucleus in parts of the brain, as well as its action on ACTH release through receptors of the adenohypophysis, can probably be numbered among the mechanisms responsible for the central effects of this hormone.

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EFFECT OF β-ENDORPHIN AND DELTA SLEEP-INDUCING PEPTIDE ON RESISTANCE TO EMOTIONAL STRESS

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Resistance to emotional stress depends on genetic and individual factors [1, 5, 8-10]. Wistar rats have been shown to be most resistant to emotional stress, whereas August rats are least resistant. Endogenous peptides (substance P, delta sleep-inducing peptide – DSIP) significantly increase the resistance of animals to emotional stress [6, 7, 9, 10]. Intravenous injection of DSIP lowers the blood pressure of spontaneously hypertensive rats [12]. Opioid peptides also have been shown to participate in the mechanisms of emotional reactions [11, 13, 14]. It is therefore interesting to study the role of endogenous peptides in mechanisms of emotional reactions in rats differing in their genetically determined resistance to emotional stress. The aim of this investigation was to determine the effect of β-endorphin and DSIP on resistance of Wistar and August rats to emotional stress.

EXPERIMENTAL METHOD

Experiments were carried out on 24 adult male Wistar and 25 adult male August rats. Resistance to emotional stress was determined in the animals of each strain by preliminary behavioral open-field testing (tail flick test) in a "Varimex" apparatus, and also by studying the character of changes in the ECG, RVG, blood pressure, and respiration rate in response to stress stimuli [4, 9, 10]. Among the animals studied, in the group of Wistar rats 13 were resistant and 11 predisposed to emotional stress. In the group of August rats 16 were predisposed and 9 resistant to emotional stress. After testing the animals were decapitated and blood and tissue from the hypothalamic regions were taken simultaneously for biochemical testing. Concentrations of immunoreactive β-endorphin-like (β-endorphin) and immunoreactive DSIP-like (DSIP) materials in acetic acid extract of blood and hypothalamus were determined by ELISA. Preliminary treatment of the blood and brain samples was carried out by the usual method [2]. Antiserum to β-endorphin and DSIP was obtained by immunizing rabbits with conjugates of β-endorphin and DSIP with hemocyanin, synthesized with the aid of carbodiimide. β-Endorphin and DSIP conjugates were emulsified in equal volumes of Freund's complete adjuvant and injected subcutaneously and intradermally at a large number of points (up to 100) over the whole body. The animals were reimmunized 2 weeks later with β-endorphin and DSIP conjugate mixed with Freund's incomplete adjuvant. Blood was collected one week after reimmunization. The peptide content was determined in the experimental samples by ELISA, using 96-well polystyrene panels ("Titertek"). To obtain quantitative information, preliminary calibration was carried

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