Identification of Neurohypophysial Hormones with Their Antisera

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ABSTRACT Identifying posterior pituitary hormones in body fluids or neurohypophysial extracts was heretofore partially achieved by using pharmacologic potency ratios or semispecific inactivation by thioglycolate or enzymes. Production of antisera against oxytocin and lysine-vasopressin has prompted us to test their specificity against lysine-vasopressin, arginine-vasopressin, arginine-vasotocin, and oxytocin. In ethanol anesthetized rats, antidiuretic and milk-ejection activities were assayed for each peptide-antiserum combination after 0, 30, 60, and 90 min of incubation. Results indicate that (*a*) oxytocin antiserum inactivates oxytocin, but not arginine-vasopressin, lysine-vasopressin, or arginine-vasotocin; vasopressin antiserum inactivates arginine-vasopressin and lysine-vasopressin, but neither oxytocin nor arginine-vasotocin; (*b*) an identifiable antigenic site exists for each hormone; (*c*) relatively specific identifications of natural neurohypophysial peptides are possible using antisera and bioassays; (*d*) this method is promising for identifying neurohypophysial peptides in body fluids and pituitary extracts; and (*e*) active and passive immunization against oxytocin and vasopressin may increase our understanding of their physiologic functions.

INTRODUCTION

Except for the *Suina,* all mammals so far studied secrete 8-arginine-vasopressin (AVP) and oxytocin from their posterior pituitary glands. In most members of the *Suina,* 8-lysine-vasopressin (LVP) replaces AVP as the antidiuretic hormone. Among nonmammalian vertebrates, almost all secrete 8-arginine-oxytocin (arginine-vasotocin, AVT) as well as oxytocin or peptides closely resembling oxytocin (1).

These neurohypophysial hormones are produced in hypothalamic nuclei, transported via axons to the posterior pituitary gland for storage and are released from there into the systemic circulation. Their basic structures are very similar, differing only in the amino acid residues at positions 3 and 8. Therefore, it is not surprising that they have similar pharmacologic effects such as antidiuretic, milk-ejection, oxytocic, avian vasodepressor, and rat vasopressor activities, or that these effects are absolutely and relatively different depending upon the structure of the peptide (2).

Naturally occurring substances other than the neurohypophysial hormones can cause some or all of the aforementioned pharmacologic effects. These substances are histamine, catecholamines, 5-hydroxytryptamine, acetylcholine, angiotensin, bradykinin, and other nonspecific peptides (3, 4). Hence, to identify tentatively a neurohypophysial peptide by its pharmacologic actions, one must use a highly purified preparation or one with so much of the hormone present that other contaminating, extraneous, pharmacologically active substances can be diluted out of consideration. However, so little hormone is present in body fluids that neither of these techniques is entirely satisfactory or possible.

For absolute identification of a neurohypophysial peptide, chemical identification of all the amino acid residues and their sequence are required, and ultimate proof requires synthesis of the proposed peptide molecule and demonstration of biological activity identical to that of the natural hormone (5). On a weight basis, the neurohypophysial hormones are the most biologically active peptide hormones known; as little as 0.01 ng of AVP or oxytocin can be detected in suitable antidiuretic or milk-ejection assays, respectively. Because of the miniscule concentration of these hormones in body fluids, chemical identification of them is impossible by currently known techniques.

Another approach used for tentative identification of the neurohypophysial hormones utilizes relatively specific destructive techniques. For example, oxytocin, but not AVP, LVP, or AVT is destroyed by chymotrypsin,

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which acts at the glycinamide-leucine bond. AVP, LVP, and AVT, but not oxytocin, are destroyed by trypsin digestion, which acts at the glycinamide-arginine or glycinamide-lysine bond. Oxytocin, AVP, LVP, and AVT are inactivated by incubation with each of the following: the oxytocinase-vasopressinase system of human pregnancy plasma, tyrosinase, and thioglycolate. These agents act by disrupting the peptide bonds between cystine and tyrosine, by the probable formation of an *O*-quinone group on tyrosine, and by disruption of the disulfide bond between the two cystine half-moieties, respectively (6). Although considerable evidence regarding structure can be adduced from such destruction experiments, a specific identification is never possible. Also, inadequate amounts of material are present for such identification experiments, especially when analyzing biological fluids such as blood plasma or urine. For these reasons simpler and more specific means of identifying neurohypophysial hormones in body fluids have been sought. Recently, antisera against LVP (7) and oxytocin (8) have been produced in rabbits and used in immunoassays for the estimation and identification of neurohypophysial hormones.

In some preliminary studies (9–11) it was found that these antisera abolished the activities of their corresponding antigenic hormones, and it also appeared that their inactivating properties were rather potent and specific. Therefore, it seemed important to evaluate the degree of structural specificity of these antisera against different neurohypophysial octapeptide substrates. Should high specificity be demonstrated, a quick and accurate test for tentatively identifying neurohypophysial peptides might be achieved.

In the present study we have established that rabbit antisera prepared against LVP and oxytocin are potent inactivators of their corresponding antigenic hormones. The specificity of these antisera was demonstrated by the absence of cross-reaction, i.e., LVP antiserum could not inactivate oxytocin and oxytocin antiserum could not inactivate LVP. Neither antiserum abolished the antidiuretic or milk-ejection activities of the structurally similar AVT.

METHODS

Bioassays. For the measurement of antidiuretic activity, ethanol anesthetized albino rats (160–230 g) under water diuresis were used. The test solutions were injected intravenously (injection volume 0.5 ml) and changes in urine flow were related to the base line flow, (average of preinjection and postresponse flow). The lowest assayable dose in this bioassay is 2.5–5 μU/dose of AVP. This assay procedure has been previously described in detail (12).

For the measurement of milk-ejection activity in ethanol-anesthetized lactating rats, a teat duct of an abdominal mammary gland was cannulated. Ipsilateral injections (injection volume 0.2 ml) were given retrograde via the femoral artery and changes in intraductal pressure were recorded. In this assay the maximal sensitivity to oxytocin is 2.5–5 μU/dose. The assay procedure used has already been described (9).

Hormone substrates. Natural AVP[1] (200, 500, and 1000 μU/ml), synthetic LVP[2] (200 and 400 μU/ml), synthetic AVT[2] (1 ng/ml), and synthetic oxytocin[2] (50, 100, and 200 μU/ml) were separately dissolved in 0.9% NaCl–0.1 M phosphate buffer, pH 7.4, and served as substrates for all incubations.

Inactivating agents. As inactivating agents, 0.01 ml of 8-lysine-vasopressin antiserum,[3] 0.01 ml of oxytocin antiserum,[4] or 0.1 ml of human near-term pregnancy plasma were used for each milliliter of substrate incubation medium. Equal volumes of normal rabbit plasma or plasma from nonpregnant women incubated with corresponding substrates served as controls.

For the production of antibodies against oxytocin and LVP, the hormones are usually coupled in vitro with carbodiimide in order to facilitate their aggregation to serum proteins and thus to enhance the formation of antibodies (7, 13, 14). Then the antigenic octapeptide is mixed with an equal volume of complete Freund's adjuvant and the emulsion is injected in the footpads of rabbits. In general, weekly doses of 0.2 mg of either oxytocin or LVP given with complete Freund's adjuvant for 3–6 wk will cause production of the respective antibodies. Additional injections in 4- to 8-wk intervals with similar hormone doses will maintain antibody formation and occasionally increase serum antibody titers.

Human pregnancy plasma and plasma of pregnant anthropomorphous apes contain a specific oxytocinase-vasopressinase enzyme system which is thought to be produced in the syncytial cells of the trophoblast. This plasma enzyme(s) increases as pregnancy advances. It is not excreted in the urine and it disappears from the bloodstream within 2–3 wk after delivery. The physiologic function of this enzyme system is not understood (6).

Inactivating capacities of these agents are shown in Figs. 1 and 2, indicating that 0.01 ml of either vasopressin or oxytocin antiserum was able to inactivate 200 μU/ml of AVP or oxytocin, respectively, within 15–20 min; 0.05 ml of human pregnancy plasma was able to inactivate the antidiuretic effect of 200 μU/ml of vasopressin within 15 min of incubation. With 200 μU/ml of oxytocin as incubation medium, 0.1 ml of human pregnancy plasma was able to destroy the milk-ejection activity after 60 min of incubation.

Incubation experiments. After 30, 60, and 90 min incubation of the various mixtures at 37°C, they were tested for antidiuretic and milk-ejection activities. These activities

[1] Purified bovine 8-arginine-vasopressin (52 pressor units/mg) was kindly supplied by Dr. F. Armstrong, Parke, Davis & Co., Detroit, Mich.

[2] Synthetic 8-lysine-vasopressin (270 pressor units/mg), synthetic 8-arginine-oxytocin (245 pressor units and 200 milk-ejection units/mg), and synthetic oxytocin (450 milk-ejection units/mg) were kindly supplied by Sandoz, Inc., Hanover, N. J.

[3] 8-Lysine-vasopressin antiserum from rabbits was prepared after the method described by Permutt, Parker, and Utiger (7) and was kindly supplied by Dr. R. Utiger, Department of Medicine, Washington University, St. Louis, Mo.

[4] Oxytocin antiserum from rabbits was prepared after the method described by Gilliland and Prout (8) and was kindly supplied by Dr. R. Bashore, Department of Obstetrics and Gynecology, University of California at Los Angeles, Calif.

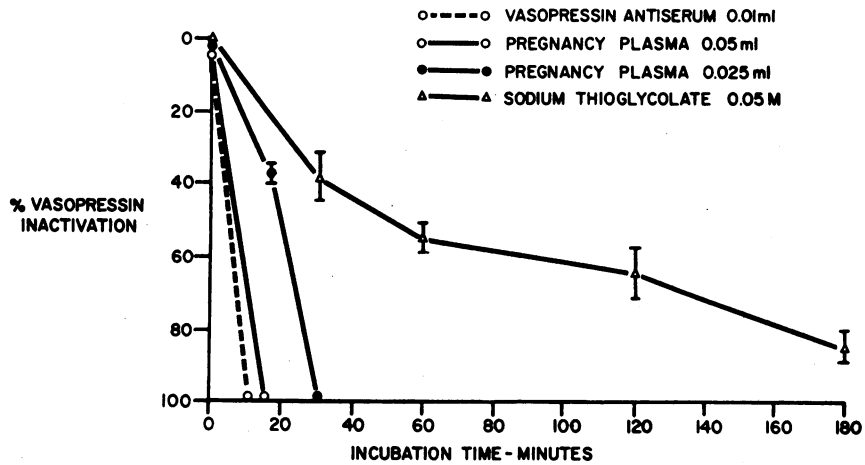

FIGURE 1 Vasopressin inactivation (rat antidiuresis assay). Inactivation of bovine arginine-vasopressin, 200 μU/ml by vasopressin antiserum, near-term pregnancy plasma, and thioglycolate. Each point represents the mean ±SE from four to seven injections in different rats.

Per cent of vasopressin inactivation =

$$\left[1 - \frac{\text{Antidiuretic activity after incubation}}{\text{Antidiuretic activity without incubation}}\right] \times 100$$

were compared with those of similar solutions of zero time incubations, i.e., inactivating agents and substrates kept separately at 37°C for 30–90 min were then mixed together and immediately injected. Antidiuretic and milk-ejection activities measured at zero time incubation were defined arbitrarily as 100% and the activities obtained at different times of incubation with different agents and media were related, percentagewise, to this base line value. Only zero time activities of corresponding AVP/oxytocin-antisera mixtures were 10–15% less than the controls with normal

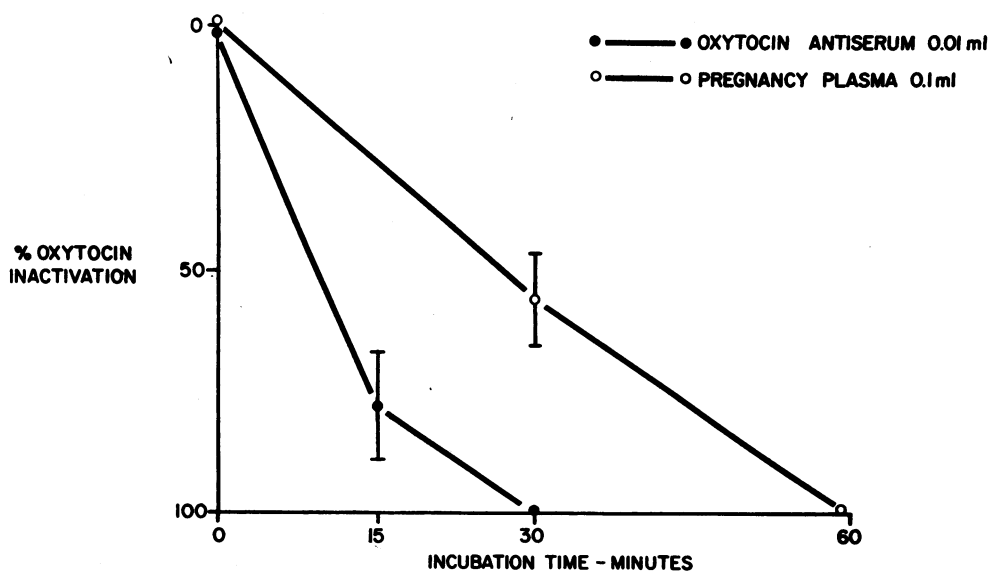

FIGURE 2 Oxytocin inactivation (rat milk-ejection assay). Inactivation of synthetic oxytocin, 200 μU/ml by oxytocin antiserum and near-term pregnancy plasma. Each point represents the mean ±SE from three to four injections in different rats.

Per cent of oxytocin inactivation =

$$\left[1 - \frac{\text{Milk-ejection activity after incubation}}{\text{Milk-ejection activity without incubation}}\right] \times 100$$

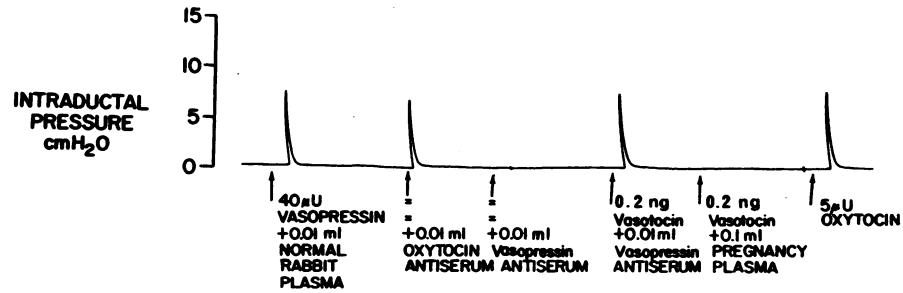

FIGURE 3 Identification of vasopressin and vasotocin (rat milk-ejection assay). Identification experiment with arginine-vasopressin and arginine-vasotocin as substrates and oxytocin antiserum, lysine-vasopressin antiserum, and near-term pregnancy plasma as inactivating agents. Vasopressin antiserum destroys the milk-ejection activity of vasopressin but not that of vasotocin. Oxytocin antiserum cannot block the milk-ejection activity of vasopressin. Pregnancy plasma eliminates the milk-ejection activity of vasotocin. Vasopressin has milk-ejection activity equivalent to 10–20% of oxytocin on a molar basis.

plasma. Also, solutions tested before and after incubation were always compared with similar antidiuretic and milk-ejection effects elicited with AVP and oxytocin standard solutions, respectively. Administration of different inactivating agents as contained in the various incubation solutions did not influence the response to subsequent injection of AVP or oxytocin standards. The latter hormones were also incubated on each assay day with their respective antiserum and tested in order to ensure the persisting potency of the antisera.

In another series of experiments 1 ml of a 1000 μU/ml solution of AVP was incubated with 0.1 ml of vasopressin antiserum and 1 ml of a 1.0 ng/ml solution of AVT was incubated separately with 0.1 ml of either vasopressin or oxytocin antiserum.

Characterization of neurohypophysial hormone-like activities in biologic fluids and neurohypophysial extracts. In this final part of the study the practical application of identifying neurohypophysial peptides was investigated. (*a*) Cerebrospinal fluid (CSF) was obtained from a dog anes-

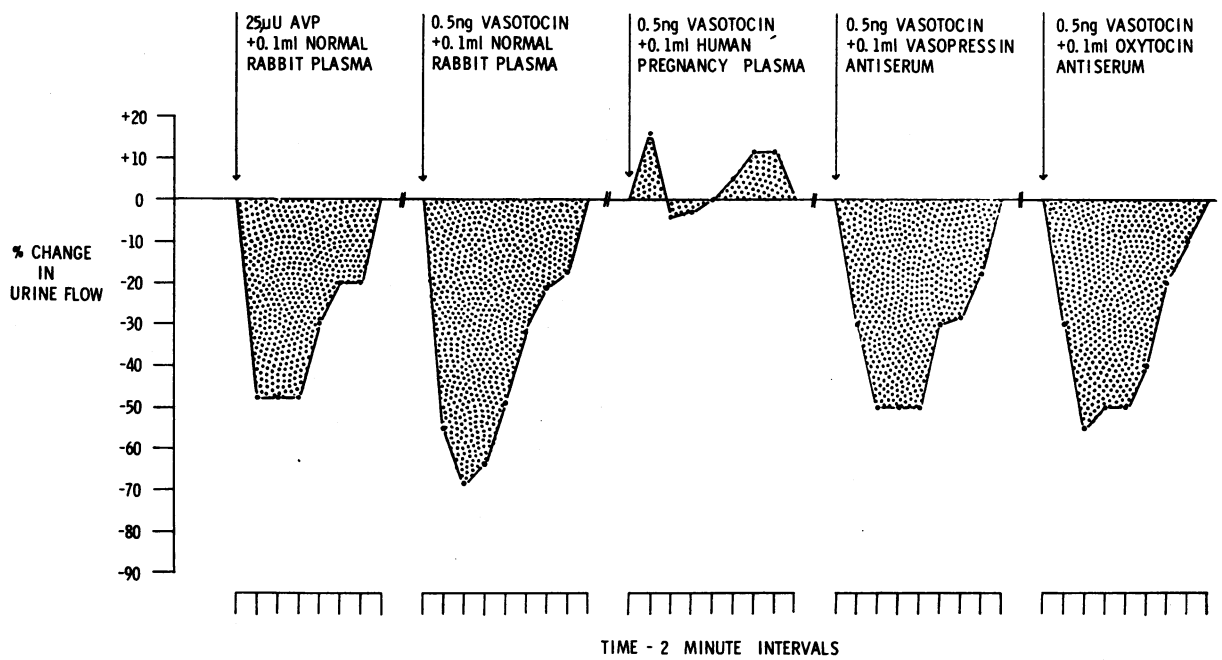

FIGURE 4 Identification experiment with vasotocin (rat antidiuresis assay). Incubation study with arginine-vasotocin as substrate, antisera and pregnancy plasma as inactivating agents. The antidiuretic activity of the different incubation mixtures is compared with bovine arginine-vasopressin. Antisera incubated with vasotocin did not alter the hormone's antidiuretic activity while after incubation with pregnancy plasma the octapeptide was inactivated.

TABLE I

Inactivation Studies with Vasopressin, Vasotocin, Oxytocin Antiserum (0.01 ml/ml of In- (0.1 ml/ml of Incubation Medium)

Substrates	Inactivating Vasopressin antiserum, Time of 0	30	60	90
8-Arginine-vasopressin				
Antidiuretic activity: 200 $\mu U/ml$	100	<5		
	[8]	[8]($P < 0.01$)		
Milk-ejection activity: 200 $\mu U/ml$	100	<25		
	[5]	[5]($P < 0.01$)		
500 $\mu U/ml$	100	<25		
	[2]	[2]($P < 0.01$)		
8-Lysine-vasopressin				
Antidiuretic activity: 200 $\mu U/ml$	100	<5		
	[3]	[3]($P < 0.01$)		
Milk-ejection activity: 400 $\mu U/ml$	100	<25		
	[5]	[5]($P < 0.01$)		
8-Arginine-oxytocin (vasotocin)				
Antidiuretic activity: 1 ng/ml	100	106 ±34		75 ±17
	[6]	[2]($P > 0.9$)		[4]($P > 0.4$)
Milk-ejection activity: 1 ng/ml	100		120 ±16	112 ±23
	[6]		[4]($P > 0.4$)	[4]($P > 0.7$)
Synthetic oxytocin				
Milk-ejection activity: 50 $\mu U/ml$	100		92 ±6	94 ±16
	[8]		[4]($P > 0.4$)	[3]($P > 0.8$)
100 $\mu U/ml$				
200 $\mu U/ml$				

Zero time activities are considered 100%. Comparative activities at different incubation times are expressed as percentage of zero time activity ±SE. Tests of significance were performed using Student's t test; values in parentheses are considered significant if $P < 0.05$. When percentage value is shown as <, this indicates that no activity was demonstrable and must therefore have been less than the threshold level in that assay. Shown in brackets is the number of animals used in each study.

thetized with sodium pentobarbital during an intravenous infusion of acetylcholine. The antidiuretic and milk-ejection activities correspond to 109 ±18 μU/ml of AVP and 69 ±8 μU/ml of oxytocin, respectively. Aliquots of this CSF were separately incubated for 30 min with 0.01 ml of oxytocin antiserum, vasopressin antiserum, and 0.1 ml of pregnancy plasma and tested again for antidiuretic and milk-ejection activity. (*b*) Blood plasma from a dog anesthetized with sodium pentobarbital was obtained during hemorrhagic shock. Plasma antidiuretic and milk-ejection activities were found corresponding to 368 ±27 μU/ml of AVP and 62 ±12 μU/ml of oxytocin, respectively. Separate aliquots of plasma were incubated for 30 min with 0.01 ml of oxytocin antiserum, vasopressin antiserum, and 0.1 ml of pregnancy plasma and tested again for antidiuretic and milk-ejection activity. (*c*) A diluted rat pituitary extract with antidiuretic and milk-ejection activities corresponding to 70 ±10 μU/ml of AVP and 30 ±9 μU/ml of oxytocin, respectively, was separately incubated with 0.01 ml of either oxytocin or vasopressin antiserum for 30 min at 37°C and then reassayed.

RESULTS

Incubation studies with vasopressin antiserum as inactivating agent. LVP antiserum (0.01 ml) completely blocked the antidiuretic and milk-ejection activity of AVP and LVP after 30 min of incubation (Table I and Fig. 3). With 0.1 ml of LVP antiserum, the activity of 1000 μU/ml of AVP was abolished after 30 min of incubation.

Neither 0.01 ml nor 0.1 ml of LVP antiserum could inactivate the antidiuretic activity of AVT or the milk-ejection activity of AVT or oxytocin (Table I, Figs.

and Oxytocin as Substrates, and with Vasopressin Antiserum, cubation Medium), and Pregnancy Plasma as Inactivating Agents

agents						
Oxytocin antiserum				Pregnancy plasma		
incubation, *min* 0	30	60	90	0	30	60
100			95 ±19	100	<5	
[4]			[4]($P > 0.9$)	[5]	[7]($P < 0.01$)	
100			102 ±33	100	<25	
[6]			[5]($P > 0.9$)	[5]	[4]($P < 0.01$)	
100			92 ±15			
[3]			[3]($P > 0.8$)			
100			101 ±7	100	<25	
[4]			[4]($P > 0.9$)	[3]	[3]($P < 0.01$)	
100		104 ±11	82 ±12	100	<5	
[6]		[3]($P > 0.8$)	[3]($P > 0.2$)	[6]	[7]($P < 0.01$)	
100		86 ±7	114 ±13	100	<25	<25
[6]		[4]($P > 0.4$)	[5]($P > 0.5$)	[7]	[4]($P < 0.01$)	[5]($P < 0.01$)
100	<25			100	<25	<25
[3]	[3]($P < 0.02$)			[6]	[3]($P < 0.05$)	[3]($P < 0.05$)
100	13 ±13			100	26 ±13	<25
[4]	[4]($P < 0.01$)			[7]	[3]($P < 0.05$)	[5]($P < 0.01$)
100	8 ±8			100	30 ±13	<25
[6]	[6]($P < 0.01$)			[4]	[3]($P < 0.01$)	[4]($P < 0.01$)

3–5). The complete inactivation of the milk-ejection activity of 1000 μU of arginine vasopressin with 8-lysine-vasopressin antiserum indicates that the purified beef vasopressin contained very little or no oxytocin. Since the milk-ejection method is sensitive to 2.5–5 μU of oxytocin per dose, the oxytocin impurity of the 1000 μU of purified beef vasopressin must lie below 12.5–25 μU.

Incubation studies with oxytocin antiserum as inactivating agent. After 30–90 min incubation of AVP, LVP, and AVT with 0.01 ml or 0.1 ml of oxytocin antiserum, no changes in hormonal activities were observed as compared with the unincubated control solutions. With 0.01 ml of oxytocin antiserum the milk-ejection activity of 50 μU/ml oxytocin was completely abolished. In three out of four experiments with 100 μU/ml and in five out of six experiments with 200 μU/ml oxytocin, complete inactivation occurred after 30 min of incubation (Table I, Fig. 5).

Incubation studies with pregnancy plasma as inactivating agent. Within 30–60 min of incubation with pregnancy plasma the antidiuretic and milk-ejection activities of AVP, LVP, AVT, and oxytocin were all destroyed (Table I, Figs. 3 and 4).

Characterization of neurohypophysial hormone-like activities in biologic fluids and hypophysial extracts. (*a*) The antidiuretic activity of CSF was eliminated after 30 min incubation with pregnancy plasma and

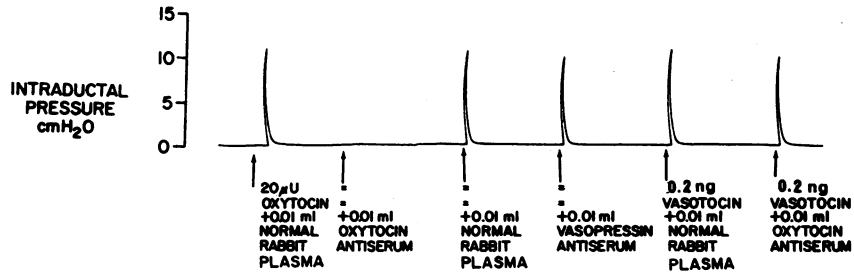

FIGURE 5 Identification of oxytocin and vasotocin (rat milk-ejection assay). Incubation study with oxytocin and arginine-vasotocin as substrates and antisera as inactivating agents. Oxytocin antiserum blocks the milk-ejection activity of oxytocin but not that of vasotocin. Vasopressin antiserum does not suppress the milk-ejection activity of oxytocin.

LVP antiserum, but not with oxytocin antiserum. The milk-ejection activity of the CSF was abolished by incubation with pregnancy plasma and oxytocin antiserum but not with LVP antiserum (Fig. 6). Therefore, we concluded that the CSF contained AVP and oxytocin. (*b*) The antidiuretic and milk-ejection activities of the plasma from a dog in hemorrhagic shock were destroyed after incubation with LVP antiserum and pregnancy plasma (Fig. 7). Incubation of the plasma with oxytocin antiserum did not influence the antidiuretic or milk-ejection activities. We therefore assume that the measured activities were due entirely or predominantly to AVP. (*c*) The antidiuretic and milk-ejection activities of the diluted rat pituitary extract were abolished only after separate incubations with LVP and oxytocin antisera, respectively. In Fig. 8 the selective suppression of the extract's milk-ejection activity after incubation with oxytocin antiserum is depicted; after incubation with LVP antiserum, the milk-ejection activity was not significantly diminished.

DISCUSSION

Chemical identification of vasopressin or oxytocin in extracted, purified, and concentrated test material is required for absolute proof of the existence of any neurohypophysial hormone (5). Since the content of neurohypophysial hormones extracted from body fluids is insufficient by present-day techniques to identify these hormones chemically, crude biologic procedures have generally been utilized.

In the present study, it was demonstrated that oxytocin, the two vasopressins, and AVT could be differentiated one from the other by incubation with vasopressin and oxytocin antisera. However, it was impossible to differentiate the two mammalian antidiuretic hormones, AVP and LVP, one from the other. With the exception of AVP, the activity of which is blocked by antibodies produced against lysine vasopressin, it was found in confirmation of earlier reports (8, 13) that antisera against vasopressin or oxytocin do not cross-react. In addition, the structurally similar AVT resisted both of these antisera even when tenfold concentrations of antisera were employed. Because LVP antiserum blocks the biologic activity of AVP and LVP, but not AVT or oxytocin, it seems that the 3-phenylalanine moiety in the ring forms a key portion of the antigenic site of the molecule and is responsible for the specificity of this antiserum. When 3-phenyl-

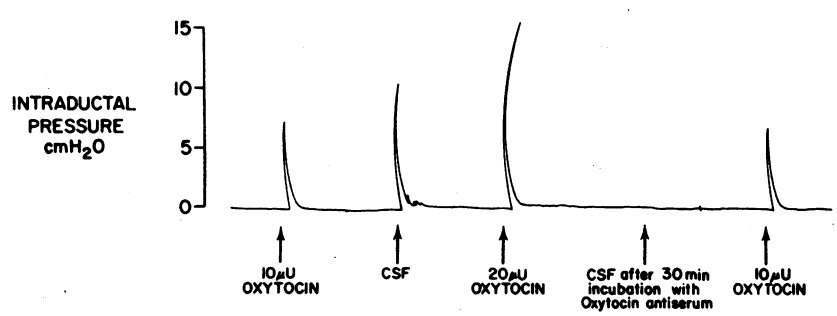

FIGURE 6 Identification of dog cerebrospinal fluid activity (CSF) (rat milk-ejection assay). The milk-ejection activity of CSF was blocked by incubation with oxytocin antiserum but not with vasopressin antiserum. This suggests that the sample contained oxytocin in measurable amounts.

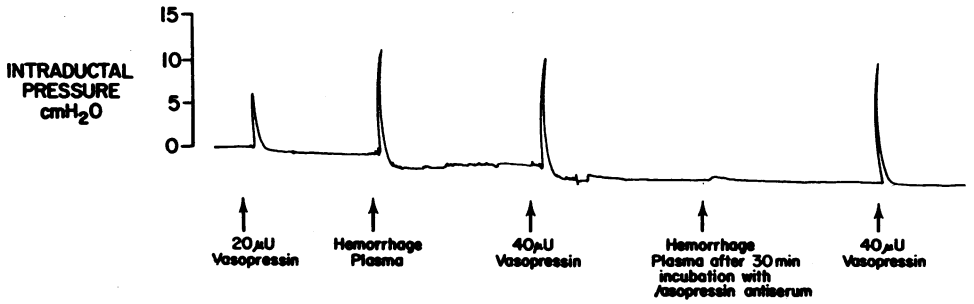

FIGURE 7 Identification of dog hemorrhage plasma activity (rat milk-ejection assay). The plasma's milk-ejection activity is destroyed after incubation with vasopressin antiserum but not with oxytocin antiserum. This suggests that the neurohypophysial hormone-like activity of the plasma is due to vasopressin.

alanine is replaced by 3-isoleucine as in AVT or oxytocin, the vasopressin antiserum fails to inhibit their biologic activities. On the other hand, oxytocin antiserum is incapable of destroying AVT, AVP, or LVP indicating that replacement of the 8-leucine moiety by arginine or lysine is sufficient to prevent inactivation by oxytocin antiserum. It is probable, therefore, that a key portion of the antigenic site of the oxytocin molecule is represented by leucine. If these hypotheses are correct, then oxypressin (2) (3-phenylalanine-oxytocin or 8-leucine-vasopressin) should be inactivated by both vasopressin and oxytocin antisera, and mesotocin (8-isoleucine-oxytocin), isotocin (4-serine-8-isoleucine-oxytocin), and glumitocin (4-serine-8-glutamine-oxytocin), all naturally occurring oxytocin-like peptides in nonmammalian vertebrates (1, 2), should be resistant to both oxytocin and vasopressin antisera. With the limited number of antisera and peptides so far studied, no sweeping conclusions can be reached concerning the manner by which certain amino acids and their locations may or may not alter antigenicity and specificity. Nevertheless, with several more hormone analogues and antisera to study, these questions should be readily answerable.

Use of antisera and biological assays does allow one to conclude that, tentatively, a biologic solution contains a definite amount of a specific neurohypophysial hormone. As demonstrated in Fig. 8, the milk-ejection activity of the rat pituitary extract was eliminated after incubation with oxytocin antiserum thus indicating that it contained oxytocin in amounts exceeding 12.5–25 μU/ml.

The tentative identification of AVP and oxytocin in CSF by using antisera and antidiuretic and milk-ejection assays provides evidence for the first time that both neurohypophysial hormones can be released into the CSF. Likewise, by incubating oxytocin and vasopressin antisera with plasma from hemorrhaged dogs, we have confirmed the observations of others (15) which suggest that under the stimulus of hemorrhage vasopressin but little or no oxytocin is released from the posterior pituitary gland into the circulation.

The rather specific behavior of these antisera suggests their potential use for the biologic identification of naturally occurring octapeptides. Despite much research it is still not clear which oxytocin-like peptides or combinations of them are secreted by various nonmammalian species (1, 16). This problem and the perplex-

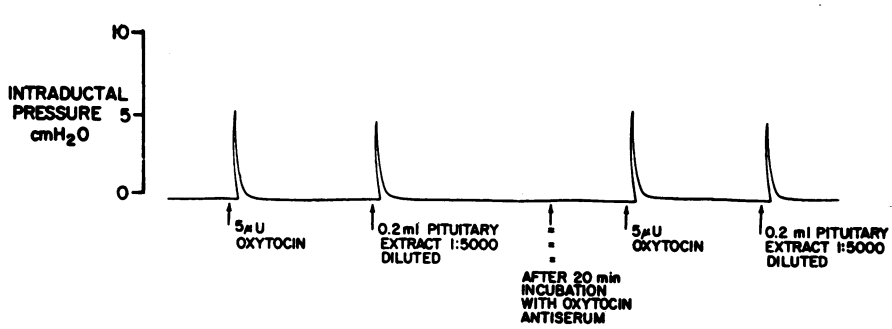

FIGURE 8 Identification of rat pituitary extract activity (rat milk-ejection assay). The extract's milk-ejection activity is blocked after incubation with oxytocin antiserum but not with vasopressin antiserum. This suggests that this sample contains oxytocin.

ing, controversial finding of AVT in extracts from beef pineal glands (17) may be assessed and reevaluated by utilizing different antisera combined with pharmacologic studies.

Utilizing suitable extraction and assay techniques combined with antiserum identification studies it seems possible now to follow more accurately the physiology and pathophysiology of neurohypophysial hormones. Also, immunization of experimental animals against neurohypophysial octapeptides may increase our knowledge in this respect. Thus, Miller and Moses (18) have recently found that active immunization of rabbits to LVP can result in anti-LVP antibody-induced diabetes insipidus. If active or passive immunization against oxytocin can be accomplished in various species, it may be possible to delineate further the effects of selective oxytocin depletion on the subsequent reproductive performance of such animals.

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