J. Physiol. (1968), **197**, *pp.* 65–76
With 4 text-figures
Printed in Great Britain

THE DISTRIBUTION OF PROTEINS THAT BIND NEUROHYPOPHYSIAL HORMONES

BY M. GINSBURG AND K. JAYASENA*

From the Departments of Pharmacology, University of Bristol, and Chelsea College of Science and Technology, University of London, London, S.W. 3

(*Received* 21 *November* 1967)

SUMMARY

1. Protein fractions were prepared from serum and various organs of pigs following the methods used to extract and purify neurophysin from posterior pituitaries.

2. Protein fractions extracted from porcine kidney, uterus, mammary gland or serum, which contain antigen reacting with anti-neurophysin serum, form non-dialysable complexes with oxytocin and/or lysine vasopressin.

3. Protein from uterus or mammary gland bound oxytocin but not lysine vasopressin, while protein extracted from kidney bound lysine vasopressin but not oxytocin: protein from serum bound both hormones.

4. Protein fractions prepared in the same way from porcine liver, spleen, skeletal muscle and brain, which do not contain antigen reacting with anti-neurophysin serum, did not form complexes with neurohypophysial hormone.

5. The formation of complexes between the renal or uterine protein fractions and lysine vasopressin or oxytocin is inhibited by the addition of $1{\cdot}0 \times 10^{-6}$ M-$CaCl_2$.

6. 1-Desamino 8-arginine vasopressin is not bound by neurophysin prepared from porcine posterior pituitaries or the protein from porcine kidneys, while 8-arginine vasopressin does form non-dialysable complexes with proteins from both sources.

7. A protein fraction extracted from guinea-pig kidney by similar preparative methods also bound lysine vasopressin and the binding was inhibited by addition of $CaCl_2$.

* Present address: Department of Pharmacology, Medical Research Institute, Baseline Road, Colombo 8, Ceylon.

INTRODUCTION

The preparation and characterization of antibody to porcine neurophysin (the carrier protein for oxytocin and vasopressin in the neurohypophysis) has been described by Ginsburg & Jayasena (1968). While the antibody showed a high degree of species specificity, protein with antigenic properties similar to neurohypophysial neurophysin was also found in extracts of porcine serum and of those organs (kidney, uterus and mammary gland) on which the principal effects of oxytocin and vasopressin are seen. Since the protein fractions used in the immunological tests were obtained by applying the same methods of gel filtration and ion-exchange chromatography used in the preparation of porcine neurophysin (Ginsburg, Jayasena & Thomas, 1966) the term 'N-fraction protein' was used to describe the proteins obtained in the final stage of preparation, from each source. The present paper is concerned with the hormone binding properties of the N-fraction proteins.

Evidence is presented to show that the distribution of neurophysin-like protein is not limited to the neurohypophysis. It is suggested that the term 'extra-neurohypophysial neurophysin' be used to describe protein with the antigenic and hormone-binding properties shown by neurohypophysial neurophysin.

METHODS

Preparation of protein fractions. Porcine neurophysin was prepared according to the methods described by Ginsburg *et al.* (1966). N-fraction proteins from porcine kidney, uterus, mammary gland, serum, brain, skeletal muscle, liver and spleen were prepared, based on the method used for the preparation of neurohypophysial neurophysin, as follows:

Porcine tissues and serum were obtained from the Slaughter Department of Walls Bacon Factory, and the guinea-pig kidneys were taken from animals at the laboratories of Parke Davis and Co. (they had been treated with egg albumin). The tissues (porcine or guinea-pig) were blended with 0·1 M acetic acid (1 l./kg net wt.) in a liquidizer (Kenwood '55'). Insoluble material was removed by centrifugation and the soluble fraction was lyophilized. The lyophilate was taken up in 0·1 M acetic acid, insoluble material again removed by centrifugation and the supernatant filtered through a Sephadex G 25 column. The high molecular weight fraction was collected, lyophilized and then taken up in 0·05 M sodium acetate (pH 4·0). This was placed in a CM cellulose column and eluted successively with 0·05 M sodium acetate buffers, pH 4·0, 4·4 and 4·8. The pH 4·8 eluate was dialysed for 18 hr against two changes of 10 l. of 0·1 M acetic acid and was then freeze-dried. The process when applied to serum was exactly the same except that the blending stage was omitted.

Assay methods. Protein was estimated by the Folin–Ciocalteau method (Lowry, Rosebrough, Farr & Randall, 1951).

Lysine vasopressin (LVP) was assayed by the blood pressure method of Dekanski (1952) using male rats anaesthetized with urethane, and treated with phenoxybenzamine. Oxytocin was assayed by the method of Holton (1948) on the isolated rat uterus suspended in Mg^{2+} free Munsick (1960) solution; 18 hr before removal of the uterus the rats were given stilboesterol.

Measurement of hormone binding. The film-dialysis technique of Craig, King & Stracher (1957) as modified by Ginsburg & Ireland (1964) was used.

The buffer used in all experiments unless otherwise stated was calcium-free modified Munsick solution pH 5·8 (Ginsburg & Ireland, 1964). In dialysis experiments involving LVP 1·0 mM L-lysine was added to the buffer solution to prevent uptake of hormone by the Visking membrane (Ginsburg *et al.* 1966).

Buffered solution containing various concentrations of hormone (usually 1·55–6·22 u. LVP/ml. or 1·32–5·28 u. oxytocin/ml.) and protein (usually 65 μg/ml.) were allowed to stand at room temperature for 15 min before 0·49 ml. of each solution was placed in the inner compartment of a dialysis cell. The inner compartment solution is separated from an outer compartment which contains 10 ml. of buffer solution, by a membrane of 18/32 in. (1·48 cm.) Visking tubing. Preliminary experiments in the absence of protein showed that the concentration of hormone in the outer compartment reached 50 % of the equilibrium values after dialysis for 19·5 and 17 min for LVP and oxytocin respectively. The solutions containing protein and hormone were dialysed for these times and the hormone content of the solution in the outer compartment was determined by bio-assay. The hormone concentration that would have been obtained in the outer compartment at equilibrium was calculated to give the free hormone concentration in u./ml. (A) and from that value and the known contents of the inner compartment before dialysis, the hormone bound in u./mg protein (R) was obtained.

Other materials. Pure LVP (260 u./mg) was kindly provided by the National Institute of Health, Bethesda, Maryland, U.S.A. 1-Desamino 8-arginine vasopressin was a sample prepared from bovine neurohypophysis, generously given by Dr B. Berde of Sandoz Ltd., Basle. The preparation of oxytocin used was Syntocinon (Sandoz) and 8-arginine vasopressin was Tonephin (Hoechst). All reagents used in the preparation of buffers, etc. were of A.R. grade.

RESULTS

Binding of hormones by N-fraction proteins from organ extracts. N-fraction protein from each organ was tested by the film dialysis method for ability to bind LVP and oxytocin. The solution dialysed (0·49 ml.) contained 32 μg of the protein fraction and 0·76–3·04 u. LVP or 0·65–2·61 u. oxytocin.

Lysine vasopressin and/or oxytocin were bound by N-fraction protein from porcine kidney, mammary gland, uterus and serum. Protein obtained from serum bound both LVP and oxytocin, while protein from kidney bound LVP but not oxytocin, and protein from uterus and mammary gland bound oxytocin but not LVP (Fig. 1). In contrast, the protein fractions obtained from porcine brain, liver, spleen and skeletal muscle did not bind significant amounts of either hormone at any of the concentrations tested (Fig. 2). Thus hormone binding protein can be extracted from the organs that are the main sites of action of the hormone, but not from those other organs tested. Moreover, the hormone binding protein fraction from each of the target organs was apparently specific for that hormone, to which the organ was the more responsive. It seemed possible that the kidney protein, for example, might yet form complexes with oxytocin but with a lower capacity and affinity than in binding LVP. Experiments were therefore carried out with oxytocin and the N-fraction protein from porcine kidney, using both the protein and the peptide in higher concentra-

tion: 4·4, 8·8 and 13·2 u. of oxytocin were dialysed in the presence of 32, 128 and 256 μg of protein. No binding of oxytocin was observed in these experiments in which the ratio of oxytocin to protein was varied within a twenty-fold range.

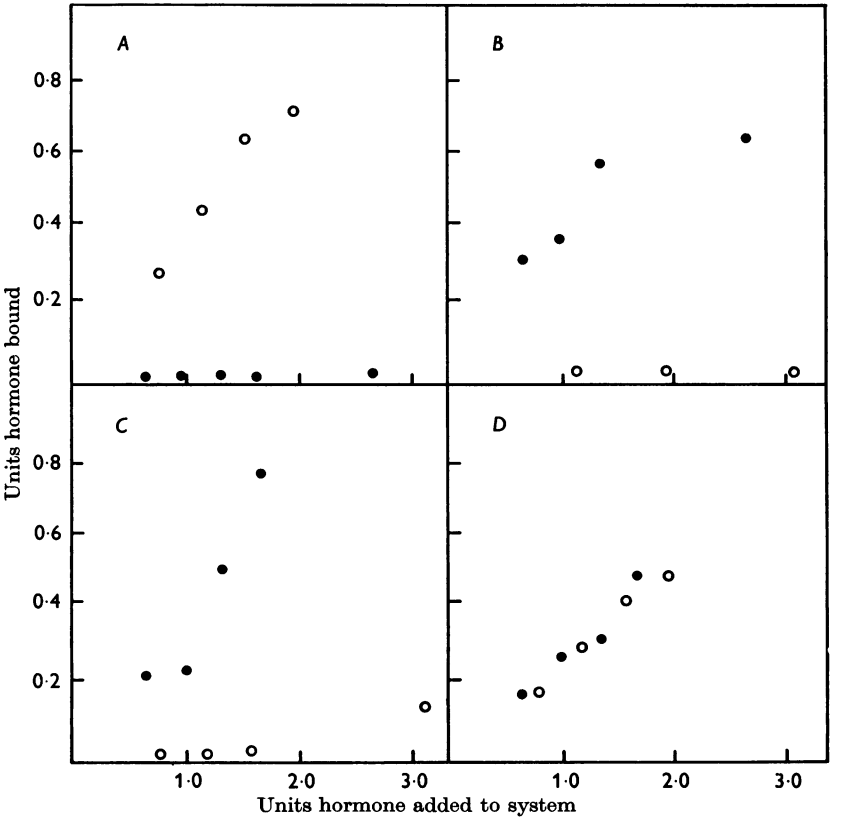

Fig. 1. Binding of lysine vasopressin and oxytocin by N-fraction protein from *A*, porcine kidney; *B*, porcine uterus; *C*, porcine mammary gland; and *D*, porcine serum. Ordinates: units of hormone bound. Abscissae: units of hormone added to the system. ● Oxytocin. ○ Lysine vasopressin.

Stability of LVP and oxytocin in the presence of N-fraction protein. Enzymatic activities that inactivate neurohypophysial hormones are known to occur in extracts of many of the organs used in the present experiments (see Heller & Ginsburg, 1966). It is clear that under the conditions of these experiments the protein fractions from liver, spleen, brain and skeletal muscle did not cause inactivation of either hormone, for otherwise the amount of free hormone recovered from the outer compartment would have been reduced. It was necessary, however, to test the

possibility that the results obtained with the fractions from the other organs, in fact, were not due to the binding of the neurohypophysial hormones but to the loss of pharmacological activity as a result of enzyme attack. Experiments were therefore carried out in which LVP was added to solutions of kidney N-fraction of domestic pig in concentrations and conditions similar to those of dialysis experiments in which the results suggested that about half of the hormone was bound. After incubation for

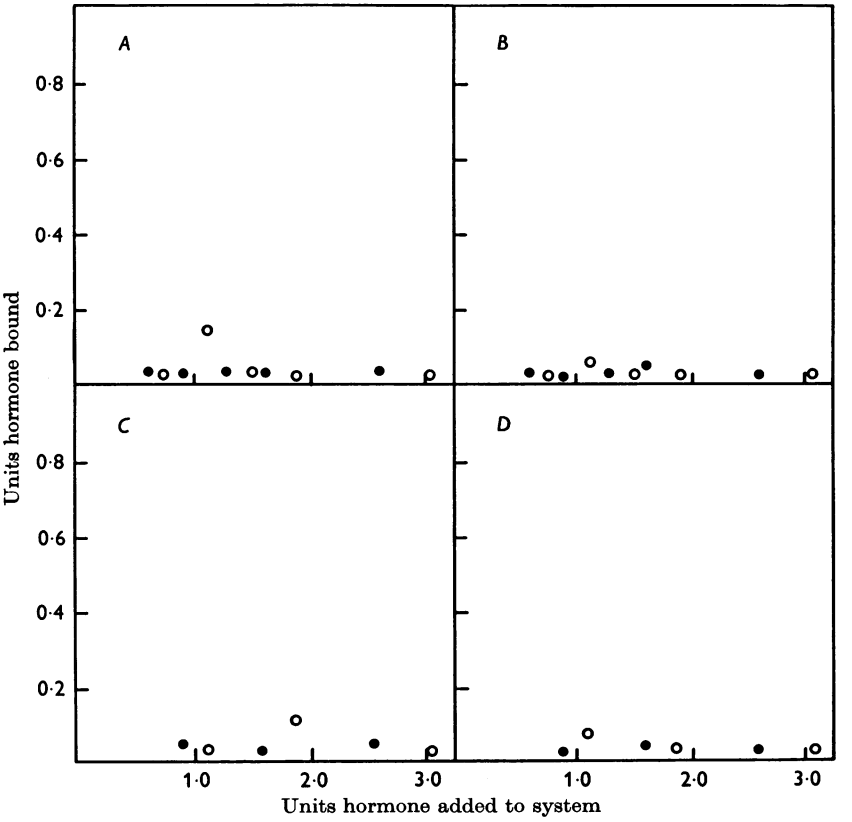

Fig. 2. The failure of 'N-fraction' protein from: *A*, porcine spleen; *B*, porcine liver; *C*, porcine skeletal muscle; and *D*, porcine brain, to bind lysine vasopressin or oxytocin. Ordinates: units of hormone bound. Abscissae: units of hormone added to the system. ● Oxytocin. ○ Lysine vasopressin.

20 min at 27° C, protein was precipitated by addition of trichloroacetic acid to a concentration of 5·0 g/100 ml.; excess precipitant was removed by treatment with Amberlite IR 45 and the LVP in the extract determined by biological assay after neutralization with NaOH. The hormone recovered was > 97 % of the amounts added and it can be concluded that

under the conditions of the dialysis experiments there was no significant destructive inactivation of LVP. In similar experiments it was shown that oxytocin could be recovered quantitatively after incubation with porcine uterine N-fraction.

Influence of calcium on N-fraction protein-hormone complexes. The formation of neurohypophysial neurophysin-hormone complexes is known to be completely inhibited in the presence of calcium in concentrations greater than 10^{-6}M (Ginsburg *et al.* 1966). N-fraction protein from porcine kidney and uterus was therefore tested for ability to form complexes in the presence of Ca^{2+} with LVP and oxytocin respectively. Dialysis of 32 μg of protein with 0·76–3·1 u. LVP, or 0·65–2·61 u. oxytocin was carried out in the presence of $0{\cdot}5 \times 10^{-6}$ and $1{\cdot}0 \times 10^{-6}$ M-$CaCl_2$ respectively. In all cases, the dialysis of hormone was as complete as in the absence of protein, indicating that the formation of a hormone-protein complex had been inhibited in the presence of $CaCl_2$. In view of this result, it was considered possible that the lack of binding by protein in extracts of spleen, liver, skeletal muscle and brain could have been due to the presence of calcium carried along with the protein during the preparatory stages. Experiments were therefore done in the presence of $1{\cdot}0 \times 10^{-5}$ M EDTA (ethylenediaminetetra-acetate), which prevents binding inhibition by added $CaCl_2$ (Ginsburg *et al.* 1966). However, even in the presence of EDTA there was no evidence for the formation of non-dialysable hormone-protein complexes.

1-*Desamino* 8-*arginine vasopressin and porcine kidney N-fraction protein.* The formation of the complex between neurohypophysial hormones and neurophysin from bovine neurohypophyses involves ionic association of the cationic free NH_2 group of the hemi-cystine residue of the peptide and anionic groups in the protein (Stouffer, Hope & du Vigneaud, 1963; Ginsburg & Ireland, 1963, 1964). 1-Desamino analogues of the hormones are not therefore bound by bovine neurophysin (Stouffer *et al.* 1963; Schwartz & Livingston, 1964) although they are often considerably more potent than the hormones from which they are derived.

Experiments were carried out to determine whether similar observations could be made with porcine neurophysin and N-fractions from porcine kidney. They were designed as comparisons of the binding of 1-desamino 8-arginine vasopressin and its natural congener 8-arginine vasopressin (AVP). Dialysis cells were loaded with 0·49, 0·98 and 1·96 u. of AVP with and without the addition of 32 μg of protein (porcine neurophysin or pig kidney N-fraction). Other cells received the desamino analogue, again with or without 32 μg of the proteins. The amounts of 1-desamino analogue were expressed as units according to the results of assay on rat blood pressure and the quantities added to the dialysis cells were again 0·49,

0·98 or 1·96 u. which are about 10 % more, in molar terms, than the same number of units of AVP.

After dialysis for 20 min peptide appearing in the diffusate was estimated by biological assay. In the absence of protein, the mean AVP content of the diffusate was 55 % (range 53–58) of the amount added; the equivalent value for 1-desamino AVP was 51 % (range 47–52). In the presence of neurophysin and kidney N-fraction, the percentage of AVP in the diffusate fell to 27 % (23–31) and 33 % (30–35) respectively. The diffusion of the 1-desamino analogue was not affected by either protein being 48 % (46–52) in the presence of neurophysin and 53 % (49–56) in the presence of kidney N-fraction.

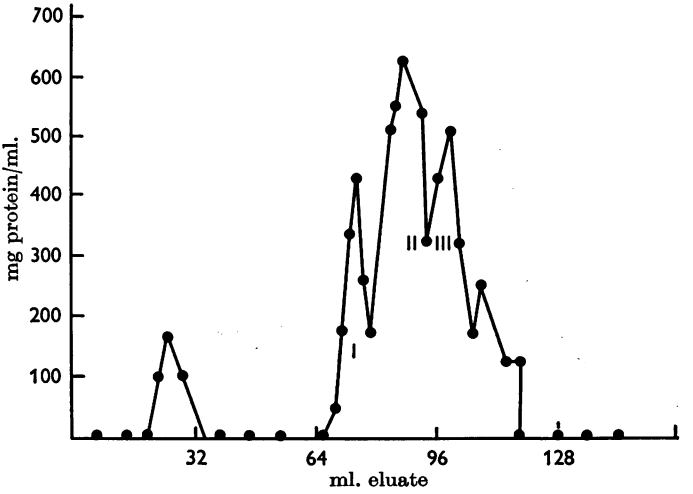

Fig. 3. Chromatography of porcine kidney 'N-fraction' protein on CM cellulose. The N-fraction protein (10 mg) was applied to the column in 0·05 M sodium acetate pH 4·0 and eluted with 0·05 M sodium acetate pH 4·8.

Further purification of porcine kidney neurophysin. The designation 'N' was applied to that fraction eluted in a batch from a CM cellulose column under specified conditions. The yield of protein in this fraction was approximately 10 mg protein/kg wt. of starting material for liver and spleen, 30 mg protein/kg wet wt. of uterus, mammary gland or kidney and 6 mg/l. of serum. No tests for homogeneity of the preparations were carried out but the presence of protein in the eluate from liver and spleen extracts suggested that the other N-fractions might also contain protein in addition to those proteins possessing the property of binding peptide hormone. An attempt was made at the further purification of N-fraction protein from porcine kidney. Figure 3 shows the elution of protein from a column of CM cellulose to which 10 mg of kidney N-fraction was applied in buffer at

pH 4·0 and eluted at pH 4·8, i.e. recycled through the final stage of the preparation of the N-fraction. There are one major and two minor peaks. The eluates were combined with three fractions corresponding to each of the peaks. The binding of LVP by the protein of the first of the minor peaks was negligible while the major peak and the second minor peak bound 17·5 and 12·1 u. of LVP/mg respectively. Although the first minor peak, being unable to bind LVP, can be clearly differentiated from the others, the separation of the fractions was incomplete and it is not possible

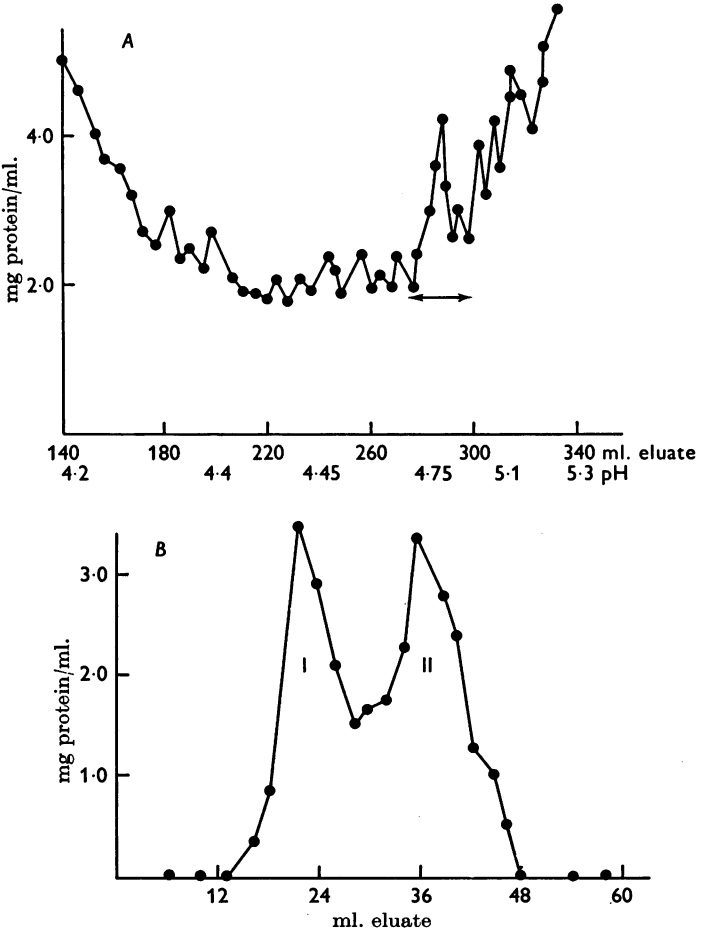

Fig. 4. *A*. Preparation of N-fraction from guinea-pig kidneys. Protein soluble in 0·1 M acetic acid was taken up in 0·05 M sodium acetate pH 4·0, placed on a CM cellulose column and eluted by a gradient of buffers to pH 5·3.

B. Chromatography of guinea-pig kidney N-fraction on CM cellulose. The protein (40 mg) was applied to the column in 0·05 M sodium acetate pH 4·0 and eluted with 0·05 M sodium acetate pH 4·8.

to state whether the major peak and the second minor peak are truly distinct or represent chromatographic artifact.

N-fraction protein from guinea-pig kidney. In the elution of N-fraction of guinea-pig kidney from CM cellulose, gradients of sodium acetate buffers pH 4·0–5·4 or pH 4·0–5·1 were used. Figure 4*a* shows the protein elution in one of those experiments and illustrates the elution of a quite definite but

TABLE 1. Binding of LVP by 'N-fraction' protein from guinea-pig kidney and by sub-fractions I and II. Varying amounts of LVP were dialysed in the presence of 32 μg of protein. *A* = free LVP, u./ml. *R* = u. LVP bound/mg protein

	N-fractions							
	Ca^{2+} free medium		$0{\cdot}5 \times 10^{-6}$M—$CaCl_2$ medium		Sub-fraction I Ca^{2+} free medium		Sub-fraction II Ca^{2+} free medium	
LVP added units	*A*	*R*	*A*	*R*	*A*	*R*	*A*	*R*
0·76	0·047	8·6	0·078	0	—	—	—	—
	0·047	8·6	—	—	—	—	—	—
1·53	0·100	15·0	0·150	0	0·10	15·0	0·13	5·6
	0·100	15·0	—	—	0·09	18·5	0·13	5·6
3·06	0·160	42·6	0·320	0	—	—	—	—
	0·150	46·2	0·320	0	—	—	—	—

small N-fraction at pH 4·75–4·85. Protein in this fraction bound LVP and the binding was inhibited completely when the buffer contained added $CaCl_2$ to the concentration of $0{\cdot}5 \times 10^{-6}$M (Table 1). LVP binding by protein eluted at pH 4·1–4·2 and at pH 4·9–5·2 was negligible. Further purification was again attempted by re-applying the N-fraction to another CM cellulose column (Fig. 4*b*). Two distinct peaks emerged and, when tested by dialysis, the protein of the first peak bound approximately 3 times as much LVP as the second (Table 1). Experiments in which LVP was incubated with guinea-pig N-fraction and subsequently extracted after protein precipitation with trichloroacetic acid showed that no degradation of the hormone had occurred.

DISCUSSION

The work described has shown the presence of protein capable of forming complexes with one or both of the neurohypophysial hormones in porcine serum and the target organs for these hormones, but not in some other organs such as brain, skeletal muscle, spleen and liver. A similar pattern was evident in the behaviour of these protein fractions as antigens in reaction with anti-neurophysin serum: protein obtained from the target organs and serum gave a positive reaction with the antiserum while protein from the other organs (i.e. liver, spleen, brain and skeletal muscle) failed to react with the antiserum (see Ginsburg & Jayasena, 1968).

Binding of vasopressin and oxytocin to plasma protein was suggested three decades ago (Heller, 1937) when it was shown that the ultra-filtrability of neurohypophysial principles was decreased when pituitary extracts were mixed with blood. More recently, it has been shown that the antidiuretic activity of serum, in rats stimulated by the intracarotid injection of hypertonic saline (Thorn & Silver, 1957) as well as exogenous hormone added to serum taken from rats under ethanol anaesthesia and therefore free from detectable endogenous hormone (Thorn, 1959), moved together with the β-globulin fraction on electrophoresis; when eluted, the hormonal activities were not ultrafiltrable. Homogenates of kidney (Dicker & Greenbaum, 1956; Heller & Zaida, 1957), liver (Heller & Urban, 1935; Birnie, 1953), mammary gland (Werle & Maier, 1954), myometrium (Sawyer, 1954; Audrian & Clauser, 1960), spleen (Christlieb, 1940), as well as cell-free extracts of liver, kidney & spleen (Heller & Urban, 1935; Miller & Townsend, 1954) have been shown to cause inactivation of neurohypophysial hormones. In most of these studies however no clear distinctions were drawn between reversible binding of the hormone and irreversible enzymatic inactivation.

The present results show that proteins obtainable from target organ sources and serum share several common properties with each other and with neurophysin obtained from the neural lobe. Thus:

(*a*) The same preparatory methods (involving gel filtration on Sephadex and ion-exchange chromatography on carboxy-methyl cellulose) yield the protein fractions from the various organs.

(*b*) The protein fractions bind either oxytocin and/or vasopressin. Evidence that the process is reversible and can be differentiated from destructive inactivation has been obtained for the N-fraction of porcine neurohypophysial protein, porcine kidney and uterus, and guinea-pig kidney. LVP or oxytocin incubated in the presence of these proteins can be recovered quantitatively after protein precipitation by the addition of trichloroacetic acid.

(*c*) The protein fractions react with antiserum raised against porcine neurohypophysial neurophysin, giving a reaction of identity in the gel diffusion test, and a single band of precipitation on immuno-electrophoresis (see Ginsburg & Jayasena, 1968).

(*d*) Fractions that have been tested so far do not form complexes in the presence of 10^{-6} M calcium, i.e. the same concentration of calcium that inhibits the formation of neurophysin hormone complexes.

(*e*) N-fraction protein from porcine kidney and porcine neurohypophysin does not form complexes with the 1-desamino analogue of 8-arginine vasopressin.

These findings are in marked contrast to the results obtained with

porcine liver, spleen, brain and skeletal muscle. The methods used in the preparation of the proteins from the target organs and serum when applied to those organs yielded small quantities of protein which did not form complexes with hormone or react with anti-neurophysin serum.

In view of the common properties shared by protein obtained from serum and target organs for neurohypophysial hormone activity, with the hormone-binding carrier protein, neurophysin present in the neural lobe, it is suggested that the term 'extra-neurohypophysial neurophysins' be used tentatively to describe these protein fractions. However, it should be mentioned that the formation of neurophysin-hormone complexes is maximal at pH 5·8 (Ginsburg & Ireland, 1964), the pH at which the present experiments were carried out, but it remains to be seen whether this is the optimal condition for hormone binding by the other proteins. The justification of the use of the term 'neurophysin' will depend on the results of these experiments and on amino-acid analyses.

At the present stage of the work it is not very profitable to consider the functional significance of these proteins. The occurrence in serum of a neurophysin-like protein capable of binding both oxytocin and LVP could be interpreted as evidence for the release of the hormones in association with the carrier protein but further evidence would be required to substantiate this conclusion. In this respect it is interesting to recall the experiments of Ames, Moore & van Dyke (1950) who showed in dehydrated dogs that urinary antidiuretic activity was associated with a macromolecule which was probably not present in urine of animals in normal water balance and that the occurrence of the macromolecule may be dependent upon stimulation of the neurohypophysis.

Since the 1-desamino analogue, which has even greater antidiuretic activity than the natural hormone, is not bound by target organ protein it is unlikely that the binding sites of the N-fraction protein could be the active receptors for pharmacological action. The observation that the protein from kidney does not bind oxytocin and that from the uterus does not bind lysine vasopressin, even though both hormones have actions on each of the two organs (antidiuretic and oxytocic actions) also seems to be incompatible with the idea that N-fraction proteins are active receptors, in whole or in part.

We wish to thank the National Institute of Health, Bethesda, Maryland, for generous supplies of LVP, Dr B. Berde of Sandoz Ltd., Basle, for a sample of 1-desamino 8-arginine vasopressin, Dr H. O. J. Collier and the staff of the Pharmacology Department at Parke Davis and Co. for supplying guinea-pig kidneys, and Mrs J. Marriott for skilful assistance.

This work was carried out during the tenure by one of us (K.J.) of a scholarship awarded by the Commonwealth Scholarship Commission, and was supported by grants from the Wellcome Trust and the Medical Research Council.

REFERENCE

AMES, R. G., MOORE, D. H. & VAN DYKE, H. B. (1950). The excretion of posterior pituitary antidiuretic hormone in urine and its detection in blood. *Endocrinology* **46**, 215–227.

AUDRIAN, L. & CLAUSER, H. (1960). Mechanisme de l'inactivation de l'ocytocine par le tissue uterin. *Biochim. biophys. Acta* **38**, 494–501.

BIRNIE, J. H. (1953). The inactivation of posterior pituitary antidiuretic activity by liver extracts. *Endocrinology* **52**, 33–38.

CHRISTLIEB, M. (1940). Uber den Abbau von Oxytocin und Vasopressin *in vitro*. *Arch. exp. Path. Pharmak.* **194**, 44–51.

CRAIG, L. C., KING, T. P. & STRACHER, A. (1957). Problem of selectivity in dialysis. *J. Am. chem. Soc.* **79**, 3729–3737.

DEKANSKI, J. (1952). The quantitative assay of vasopressin. *Br. J. Pharmac. Chemother.* **7**, 567–572.

DICKER, S. E. & GREENBAUM, A. L. (1956). Inactivation of antidiuretic activity of vasopressin by tissue homogenates. *J. Physiol.* **132**, 199–212.

GINSBURG, M. & IRELAND, M. (1963). The hormone binding proteins of the neurohypophysis. *J. Physiol.* **169**, 15*P*.

GINSBURG, M. & IRELAND, M. (1964). Binding of vasopressin and oxytocin to protein in extracts of bovine and rabbit neurohypophyses. *J. Endocr.* **30**, 131–145.

GINSBURG, M. & JAYASENA, K. (1968) The occurrence of antigen reacting with antibody to porcine neurophysin. *J. Physiol.* **197**, 53–63.

GINSBURG, M., JAYASENA, K. & THOMAS, P. J. (1966). The preparation and properties of porcine neurophysin and the influence of calcium on the hormone-neurophysin complex. *J. Physiol.* **184**, 387–401.

HELLER, H. (1937). The state in the blood and the excretion by the kidney of the antidiuretic principle of posterior pituitary extracts. *J. Physiol.* **89**, 81–95.

HELLER, H. & GINSBURG, M. (1966). Secretion, metabolism and fate of the posterior pituitary hormone. In *The Pituitary Gland*, vol. 3, ed. HARRIS, E. N. & DONOVAN, B. T. London: Butterworths.

HELLER, H. & URBAN, F. F. (1935). The fate of the antidiuretic principle of posterior pituitary extract *in vivo* and *in vitro*. *J. Physiol.* **85**, 502–518.

HELLER, H. & ZAIDA, S. M. A. (1957). The metabolism of exogenous and endogenous antidiuretic hormone in the liver and kidneys *in vivo*. *Br. J. Pharmac. Chemother.* **12**, 284–292.

HOLTON, P. (1948). A modification of the method of Dale and Laidlaw for the standardization of posterior pituitary extract. *Br. J. Pharmac. Chemother.* **3**, 328–334.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin-phenol reagent. *J. biol. Chem.* **193**, 265–275.

MILLER, G. E. & TOWNSEND, C. E. (1954). The *in vitro* inactivation of Pitressin by normal and cirrhotic livers. *J. clin. Invest.* **33**, 549–554.

MUNSICK, R. A. (1960). Effect of magnesium ion on the response of the rat uterus to neurohypophysial hormones and analogues. *Endocrinology* **66**, 451–457.

SAWYER, W. H. (1954). Inactivation of oxytocin by homogenates of uteri and other tissues from normal and pregnant rats. *Proc. Soc. exp. Biol. Med.* **87**, 463–465.

SCHWARTZ, I. L. & LIVINGSTON, L. (1964). Effects of structural deletions on the activity of lysine vasopressin. *J. clin. Invest.* **43**, 1267.

STOUFFER, J. E., HOPE, D. B. & DU VIGNEAUD, V. (1963). In *Perspectives in Biology*, ed. CORI, C. F., FOGLIA, V. G., LELOIR, L. F. & OCHOA, S., pp. 75–80. Amsterdam: Elsevier Publ. Co.

THORN, N. A. (1959). Binding *in vitro* of highly purified arginine vasopressin and synthetic oxytocin to rat serum globulin. *Acta endocr., Copenh.* **30**, 472–476.

THORN, N. A. & SILVER, L. (1957). Chemical form of circulating antidiuretic hormone in rats. *J. exp. Med.* **105**, 575–583.

WERLE, E. & MAIER, L. (1954). Uber den Abbau von Oxytocin und Vasopressin durch Milchdrusenextrakte der Kuh. *Naturwissenschaften* **41**, 480.