AGENTS DETERMINING AND INFLUENCING THE FUNCTIONS OF THE PARS NERVOSA OF THE PITUITARY*

BY

E. B. VERNEY, F.R.S., F.R.C.P.

Professor of Pharmacology, University of Cambridge

The pars nervosa of the pituitary is essentially the macroscopic representation of the multiple and terminal branching of axons most of which originate from the supraoptic nuclei. These nuclei are bilaterally placed in the ventral surface of the anterior part of the hypothalamus; each is separated into an anterior and posterior division by the emergence of the optic tract from the optic chiasma, and the axons stream thence, as the supraoptico-hypophysial tracts in the antero-ventral wall of the infundibulum, to their distribution in the pars nervosa. The structural entity comprising the nuclei, tracts, and pars nervosa is called the neurohypophysis. Extracts of the pars nervosa exhibit anti-diuretic, oxytocic, and, under certain experimental conditions, pressor and diuretic properties, and I propose in this opening paper to confine consideration to the first of these by briefly reviewing the evidence which has led to the endowment of this pharmacological property with the significance of a physiological function.

Conditions Leading to Profuse Watery Diuresis

There are three conditions under which a profuse watery diuresis is observed, and in each instance the effect of posterior pituitary extract is to reduce the rate of water

and, thirdly, the administration by mouth of large volumes of water to the living mammal.

The effects of post-pituitary extract on the polyuria associated with the first condition are closely simulated by switching into the perfusion circuit, in parallel with the kidney, the head of a dog, the inhibition of diuresis and

FIG. 1.—Inhibitory responses to intracarotid injection of hypertonic solution during water diuresis in the dog. *a*: at *A*, injection of 10.5 ml. of 1.50% NaCl into left carotid in 9 seconds. *b*: at *B*, injection of 11 ml. of 1.50% NaCl into right carotid in 13 seconds. Abscissae: time after the test dose of water.

output by the kidney and to increase the percentage of chloride in the urine. These conditions are, first, perfusion of the dog's kidney in the isolated state; secondly, experimental or pathological lesions of the neurohypophysis;

FIG. 2.—Responses to injections: *a* and *b* 5 days before, *c* and *d* 11 days after, removal of posterior lobe of pituitary. *a*: at *A*, injection of 21 ml. of 2.50% NaCl into right carotid in 20 seconds. *b*: at *A*, 1 mU of post-pituitary extract injected intravenously in 15 seconds. *c*: at *A*, injection of 21 ml. of 2.50% NaCl into right carotid in 20 seconds. *d*: at *A*, 0.1 mU (curve *C*) and 0.2 mU (curve *D*) post-pituitary extract injected intravenously in 20 seconds. Abscissae: time (approx.) after the test dose of water.

increased excretion of chloride being then dependent upon the presence of the pituitary in the perfused head; and these effects may occur without appreciable diminution of blood flow through the kidney at constant perfusion pressure. The view was put forward, therefore, that the phenomenon of profuse watery diuresis exhibited by the kidney perfused in the isolated state was due to the divorce of the kidney from the inhibitory influence of the antidiuretic substance elaborated in the pars nervosa.

This view has received strong support from observations made under the second of the conditions to which I have just referred—viz., experimental or pathological lesions of the neurohypophysis. The chequered aetiological history

*Read in opening a discussion in the Section of Pharmacology at the Annual Meeting of the British Medical Association, Cambridge, 1948.

FIG. 3.—The injections were made at the arrow. The corresponding changes in urine chloride are given by the curves at the bottom of the figure. Abscissae: time after the test dose of water.

of the diabetes insipidus associated with these has culminated in the elegant demonstration by Ranson and his colleagues that the occurrence of diabetes insipidus is contingent upon the complete or quasi-complete degeneration or removal of the neurohypophysis. Using the Horsley-Clarke stereotaxic instrument, they have been able to place small discrete lesions at any desired points in the hypothalamus, and to show that when by these means the supraoptico-hypophysial tracts are interrupted a series of striking degenerative changes takes place in the supraoptic nuclei and in the neural division of the hypophysis, the degeneration being associated with a fall of some 95% in antidiuretic activity as measured by comparative assays of extracts of the normal and of the atrophic pituitaries. The view, therefore, that diabetes insipidus derives essentially from a deficiency, complete or extreme, in the antidiuretic product of the neurohypophysis would appear, on the available evidence, to be incontrovertible.

The third condition under which a profuse watery diuresis appears is the administration of water by mouth to the living mammal, and the question arises whether this phenomenon, too, is of pituitary origin. When water is given by stomach-tube to the dog under conditions which, both externally and internally, are kept as constant as possible, the diuretic responses to successive doses are sufficiently uniform to allow analysis of the phenomenon's underlying events. That such responses are not so simply determined as appearance suggests is shown by the fact that the course of alimentary absorption is well in advance of that of renal excretion, there being in the dog, under certain experimental conditions, an interval of 15 minutes between the peak of the water-load—i.e., the proportion of absorbed but as yet unexcreted water—and the peak of diuresis. The significance of this interval will be considered later. The response of the innervated kidney runs strictly parallel with

that of the denervated kidney, and the response is unaccompanied by any change in the volume flow of blood through the kidney.

Inhibition of Diuresis Response

Next, this diuresis response may be inhibited in various ways—for example, by subjecting the animal to short-lived muscular exercise or to emotional stress, and by intravenous injections of adrenaline or of post-pituitary extract. The inhibition produced by short periods of muscular exercise in the dog—e.g., running at 6 m.p.h. for 4 minutes—has been shown to be due to the emotional accompaniment of the exercise; and, since the inhibition from emotional stress was very much like that produced by an intravenous dose of post-pituitary extract, it seemed worth while to analyse the phenomenon with some care. The induced emotional disturbance need be a very small one, an unfamiliar and ugly sound being sometimes enough to elicit

an inhibitory response. Usually, however, a weak faradic current carried to the subcutaneous tissues by surgically clean needle electrodes, as being a simpler and more controllable form of stimulus, has been used for the analysis of the resultant inhibition. From such analysis we now know that the inhibition of water diuresis by emotional stress is independent of the nerve supply to the kidney, is not caused by the endogenous release of adrenaline, is unaccompanied by any appreciable change in the volume flow of blood through the kidney, and is deferred by some two minutes from the time of the stimulus. The inhibition of water diuresis by the intravenous injection of post-pituitary extract likewise shows all these characteristics, and with a suitable dose of extract the response to the one agent is indistinguishable from that to the other.

So close a resemblance between the two responses, both in form and in measurable accompaniments and independencies, made one confident that removal of the posterior lobe of the pituitary would be followed by a big reduction in the inhibitory response to emotional stress. This was found experimentally to be so. There can, then, be little doubt that the stimulus produces the observed changes in the excretion of water and chloride by ultimate involvement of the supraoptic group of hypothalamic cytons whose axons pass down the stalk of the pars nervosa, and by whose activity the pituitary antidiuretic substance is released. Certain pharmacological agents, too, affect the functional activities of the neurohypophysis.

A Controlling Factor

The demonstration in the normal animal of the release of pituitary antidiuretic substance by the artificial means of faradic stimulation of sensory nerves and receptors, and the fact that this release may be caused by such mild disturbance of the central nervous system as comes within

a physiological range, encouraged an attempt to determine whether the secretion of this substance was not continuously varying with, and under the direct control of, some factor in the animal's internal environment, to the maintenance of which factor within a narrow physiological range the renal excretion of water and of chloride would specifically contribute. Such a factor is clearly the osmotic pressure of the arterial blood, and it became of interest to determine the effects of a rise in this pressure on the secretion of the kidney during water diuresis.

For this purpose dogs have been provided with carotid loops whereby injections or infusions may be made direct into the common carotid blood stream. While intracarotid injection of isotonic solution of sodium chloride and intravenous injection of hypertonic solutions are without apparent action on the course of water diuresis, intracarotid injection of hypertonic solutions causes definite inhibitory responses. They are illustrated in Fig. 1. The shape of these responses suggested that they were of pituitary origin, and this hypothesis was put to the test of experiment by measuring the response to a given injection before and after removal of the posterior lobe. Fig 2a gives the response to 21 ml. of 2.50% NaCl injected into the right carotid in 20 seconds, and we may note in passing that it equates closely with the response to 1 mU of post-pituitary extract injected intravenously (Fig. 2b).

The response to the same intracarotid injection after removal of the posterior lobe is given in Fig. 2c: it is very much reduced in size. It is therefore a valid procedure to assay the responses in terms of post-pituitary extract; and when this is done it is found that the response to a given intracarotid injection is diminished by some 90% as the result of removal of the posterior lobe: Fig. 2d gives the response to an intravenous injection of 0.1 mU.

Action of Sodium Chloride

It now became important to know whether the sodium chloride was acting specifically or by virtue of the increase which it produced in the osmotic pressure of the plasma. To this end equal increases in the osmotic pressure of the carotid blood were produced by injections of sodium chloride, sodium sulphate, dextrose, and urea. While the responses to sodium chloride and sodium sulphate were indistinguishable, the response to dextrose was seemingly a little smaller, suggesting that the osmotic effectiveness of dextrose was less than that of the other two solutes. Urea, on the other hand, was found to be quite inactive. A similar comparison was then made between the effects of osmotically identical injections of sodium chloride and sucrose. The results are given in Fig. 3. Three responses are shown: two of them (black circles and squares) are to the injection of the sodium chloride solution, and the third (open circles) is to the injection of the sucrose solution. The calculated percentage increases in the osmotic pressure of the carotid blood during the injections are 52 and 53

FIG. 4.—The rectangle A covers the period of the infusions. Abscissae: time (approx.) after the test dose of water.

respectively. The response to sucrose lies between the two responses to sodium chloride. Moreover, the courses of the concentration of chloride in the urine (shown at the bottom of the figure) are identical. Seeing that the only common and equal change in property of the carotid blood caused by the injection of these two solutions is the increase in osmotic pressure, and that both produce quantitatively the same release of post-pituitary antidiuretic substance, the osmotic determination of the phenomenon is beyond cavil.

"Osmoreceptors"

It becomes justified, therefore, to introduce the term "osmoreceptors" as descriptive of the autonomic receptive elements with which the neurohypophysis is functionally linked and through whose activation the pituitary antidiuretic substance is released; and the facts so far presented show that when they are exposed to short-lived and large increases in the osmotic pressure of their environment their "membrane" is relatively impermeable to sodium chloride, sodium sulphate, and sucrose, less impermeable to dextrose, and freely permeable to urea.

Where, then, do these receptors lie? They are not in the carotid sinus nor in the carotid body. Comparison of the effects of like increases in the osmotic pressure of the common carotid blood before and after ligation of the internal carotid artery, however, has shown that such ligation virtually abolishes the response. The receptors lie, therefore, in the vascular bed normally supplied by the

FIG. 5.—Effect of 40-minute infusion of NaCl into the carotid, and the method of its assay. The black rectangle covers the period of the infusions. With the intracarotid infusion the calculated local increase in osmotic pressure is 1.8%. Abscissae: time (approx.) after the test dose of water.

internal carotid artery. Their precise location and histological identification must be left to future experimentation.

So far we have been considering the responses of the osmoreceptors to short-period (10 to 20 seconds) and large (50 to 100%) increases in the osmotic pressure of their environment. It now became of interest to determine the effects of exposure of the receptors to much smaller increases maintained over longer periods. For this purpose intravascular infusions of various solutes have been made at uniform rates over periods of 10 and 40 minutes. It was found that when over a period of 10 minutes a hypertonic solution of sodium chloride was infused into a carotid the inhibition of urine flow occurred earlier than when the same infusion was made into the malleolar vein, and that as the strength of the infused solution was reduced this difference became greater. The cause of the earlier onset of the inhibition when the infusion was made into the carotid must be attributed to the increase in osmotic pressure of the internal carotid blood over and above that of the aortic blood.

This difference between the effects of intracarotid and intravenous infusions is illustrated in Fig. 4. During the 10-minute period shown by the black rectangle A sodium chloride was being introduced into the circulation at the rate of 1 mg./sec. The effect of this procedure on the course of the urine flow when the infusion was made into the right carotid is shown by the black circles, when into the malleolar vein by the open circles. The third curve (black-and-white circles) shows that the response to the intracarotid infusion is closely matched when to the intravenous infusion is added post-pituitary extract in such amount that 1.67 μ U is being introduced into the circula-

tion every second. The calculated local increase in osmotic pressure produced by the intracarotid infusion is 5.4%. Comparable experiments with sodium sulphate and with sucrose gave results which on assay were closely similar to those of the sodium chloride experiments. With dextrose, however, two striking differences appeared: there was a small response to the intravenous infusion; and the response to the intracarotid infusion, though larger than that to the intravenous, was definitely smaller than that to an osmotically equivalent infusion of sodium chloride. The osmoreceptors therefore are, under these conditions, at least partially permeable to dextrose.

Long-period Intracarotid Infusion

We come now to the effects of a much smaller increase in the osmotic pressure of the carotid blood maintained over a much longer period—

viz., 40 minutes. The results obtained with infusions of sodium chloride at 0.33 mg./sec. are illustrated in Fig. 5. The black rectangle covers the period of the infusions. With the intracarotid infusion (curve A, black circles) the urine flow, after a latent period of some 10 minutes, begins to fall; eventually it reaches the low rate of 0.5 ml./min., at which level the flow is persisting when the infusion is stopped. The flow then slowly increases, to reach a rate of nearly 4 ml./min. thirty minutes later. The intravenous infusion (curve E), on the other hand, has no appreciable influence on the course of the diuresis. The calculated percentage increase in the osmotic pressure of the carotid blood during the intra-arterial infusion is 1.8, corresponding with a local increase in blood chloride of 9 mg./100 ml., and the experiment shows that under these conditions the "membrane" of the osmoreceptors maintains its relative or complete impermeability to sodium chloride for a period of at least 40 minutes.

In strictly comparable experiments with dextrose, however, no difference was detected between the effect of the intracarotid and that of the intravenous infusion. With the intracarotid infusion the local increase in blood dextrose responsible for the 1.8% increase in osmotic pressure is some 90 mg./100 ml.; the conclusion is justified, therefore, that the "membrane" of the osmoreceptors is permeable to dextrose to such degree that a maintained rise in blood dextrose of some 90 mg./100 ml.—a rise which, incidentally, is within the human pathological range—fails to elicit an antidiuretic secretory response by the neurohypophysis. Patients with diabetes mellitus are still permitted to be polyuric. When similar experiments were made with sucrose the results of the intracarotid infusions showed

close quantitative agreement with those obtained with sodium chloride. The assay of such response to sodium chloride is illustrated in Fig. 5. The response to the intracarotid infusion (black circles) lies for most of its course between the responses to 0.67 (black-and-white circles) and 1.17 (open circles) $\mu\text{U}/\text{sec.}$, being nearer to and probably a little less than the latter: it is assayed as having a post-pituitary-extract equivalence of 1 $\mu\text{U}/\text{sec.}$

Conclusions from Results of Long-period Infusions

Three conclusions follow from the results of the 40-minute infusions. First, that increases in the osmotic pressure of the arterial blood—increases which, when large, were shown by the short-period injections to release post-pituitary antidiuretic substance—are still operative when they are reduced to values well within a range which may reasonably be regarded as physiological. An increase of only 1.8% in the osmotic pressure of the carotid blood gradually reduces the rate of urine flow from a water-diuresis maximum to the sort of rate which prevails at the beginning and end of a normal response to ingested water—i.e., a reduction of some 90%. The smallness of the osmotic pressure increase gains additional interest when it is recollected that the intracarotid infusions were unilateral. For if, as is probable, only half of the total number of osmoreceptors are being exposed under these conditions to the osmotic pressure increment, an increase of some 1% only (54 mm. Hg in absolute terms) in the osmotic pressure of the aortic blood would suffice to produce the same degree of inhibition of urine flow—i.e., a reduction to about 10% of the maximum rate of which the kidney is capable during water diuresis.

Secondly, the results of the 40-minute infusions demonstrate that the induced change in the osmotic pressure of the arterial blood which is responsible for this degree of reduction in urine flow itself causes the release of post-pituitary antidiuretic substance at an average rate of 1 $\mu\text{U}/\text{sec.}$ (0.5×10^{-9} g./sec. in terms of the standard powder), this being the intermediating agency through which the change in osmotic pressure becomes effective.

Thirdly, the recovery of urine flow when the intracarotid infusion is stopped (Fig. 5) shows that the secretion of post-pituitary antidiuretic substance is now inhibited by the local fall in osmotic pressure and consequent depression of activity in the osmoreceptors, the progression of this recovery being attributable to the gradual destruction in the kidney, and maybe in the blood, of the quantity of antidiuretic substance which was maintaining the secretion of urine at a non-diuretic level. The latent period between the peak of the water-load curve and the maximum rate of urine secretion, to which I referred earlier and promised to return, is clearly to be attributed to the same process.

Water diuresis, then, is fitly and accurately described as a condition of physiological diabetes insipidus, and there can be little doubt that the antidiuretic secretion of the neurohypophysis is a hormone in the physiological sense that its liberation is mainly and continually governed by the contemporary concentration of sodium chloride in the carotid arterial plasma. The physiological fitness of this control is emphasized by its quantitative aspects, in that changes within the range and of the order of 1% in the osmotic pressure of the arterial blood lead, through the intermediation of the antidiuretic hormone, to changes in the rate of water excretion within the range and of the order of 1,000%: the maintenance of near constancy in the osmotic pressure of the internal environment is thereby achieved.

THE USE OF POST-PITUITARY EXTRACT IN PHYSIOLOGICAL AMOUNTS IN OBSTETRICS

A PRELIMINARY REPORT

BY

G. W. THEOBALD, M.D., F.R.C.S.Ed., F.R.C.O.G.

A. GRAHAM, M.B., Ch.B.

J. CAMPBELL, M.B., Ch.B.

*P. D. GANGE, M.R.C.S., L.R.C.P.

AND

W. J. DRISCOLL, M.B., B.Ch.

(From the Obstetric Unit, St. Luke's Maternity Hospital, Bradford)

In 1895 Oliver and Schäfer reported that extracts made from the pituitary gland caused a rise of blood pressure in anaesthetized animals when injected intravenously. Three years later Howell (1898) demonstrated that their pressor activity was confined to the posterior lobe, and this was confirmed in the following year by Schäfer and Vincent. In 1901 Schäfer and Magnus reported that post-pituitary extract exerted both diuretic and antidiuretic activities, and it was not until 1915 that Konschegg and Schuster first demonstrated that in conscious animals only an antidiuretic response could be obtained. Dale, in 1906, was the first to draw attention to the fact that this extract stimulated uterine muscle, and this activity was confirmed in 1909 by Frankl-Hochwart and Fröhlich.

It is now generally accepted that post-pituitary extract contains two active principles: (a) a pressor which is identical with the antidiuretic activity, and (b) an oxytocic substance. Both principles are destroyed by heat, tryptic digestion, hydrochloric acid decomposition, and alkaline hydrolysis, and both are of approximately the same molecular size. Further, in the most purified extracts the pressor and oxytocic activities remain in the same proportions as in an ordinary post-pituitary extract. It was for these reasons that for many years controversy raged on whether post-pituitary extract contained one or more active principles, and it was not until 1928 that Kamm *et al.* settled the dispute by separating and concentrating two active principles in the form of potent solid preparations.

In 1925 a Committee of the League of Nations adopted the U.S.P. Standard Reference Powder as the international standard, 0.5 mg. of this powder being equivalent to one international unit. The international standard preparation of the post-pituitary gland is simply a powder made from the whole posterior lobes of cattle, collected immediately after death and ground in acetone in order to remove water and fat. The different standard powders may therefore differ somewhat in activity, but it is unlikely that the discrepancy ever exceeds 20% in either direction.† Until 1928 post-pituitary extracts were standardized for their oxytocic activity by a method devised by Dale and Laidlaw in 1912, but the separation of two distinct principles by Kamm *et al.* suggested the desirability of devising a separate method for assaying the pressor activity of these extracts.

Blair Bell was the first to use post-pituitary extract in obstetric practice, and in 1909 he reported its efficacy in the treatment of post-partum haemorrhage and intestinal atony. It is of interest to note that before this date no clinical application of

*Mr. Gange terminated his appointment as resident obstetric officer at the end of December, 1947.

†We are greatly indebted to the Director of the Department of Biological Standards, the National Institute for Medical Research, Hampstead, for the above information.