J. Physiol. (1966), **182**, *pp.* 217–231
With 7 text-figures
Printed in Great Britain

ACTIVITY OF NEURONES IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS AND ITS CONTROL

BY C. McC. BROOKS, T. ISHIKAWA, KIYOMI KOIZUMI AND H-H. LU*

From the Department of Physiology, State University of New York Downstate Medical Center, Brooklyn, New York, U.S.A.

(*Received* 27 *April* 1965)

SUMMARY

1. Activity of the paraventricular nucleus neurones was recorded by micro-electrodes during resting conditions and while various osmotic, chemical, direct and indirect neural stimuli were applied. This activity was correlated with evidence of oxytocin release by recording milk ejection responses.

2. Seventy-four per cent of all paraventricular nucleus neurones were osmosensitive in that firing was augmented following close arterial injection of hypertonic solutions (1 ml. 1 M-NaCl in 10–15 sec). A very few neurones showed decreased activity and in twenty-two per cent no change at all occurred following injection. Evidence indicated that the observed depressions of cellular firing rate were due to other than osmotic stimulation.

3. In post-partum cats an osmotically-induced neurone discharge increase was accompanied by a milk ejection response equivalent to that produced by 2–3 m-u. of oxytocin.

4. Paraventricular neurones were also sensitive to acetylcholine. Intracarotid injections of 40–80 μg of acetylcholine greatly increased discharge rates and caused a very definite milk ejection response.

5. Stimulation of the nipples by gentle suction, but not by electrical shock, and distension of the uterus in post-partum cats increased unit discharge in the paraventricular nucleus and evoked a milk ejection response.

6. Neurones of the paraventricular nucleus, unlike those of the supraoptic nucleus, did not appear to be specifically responsive to electrical stimulation of skin and muscle afferent or of central nervous structures. Such stimuli did cause slight augmentations or depressions in firing rates of cells within and adjacent to the paraventricular nuclei, but many neurones were unaffected. Stimuli such as those applied appeared to have

* Fellow of the China Medical Board of New York, Inc.

a general central effect involving paraventricular as well as many other neurones.

7. Direct electrical excitation of the pituitary stalk produced a milk ejection response in post-partum cats. Electrical pulses applied to the paraventricular nuclei were less effective for reasons discussed.

INTRODUCTION

It has been well established in recent years that the paraventricular nuclei of the hypothalamus are concerned with production and release of oxytocin from the neurohypophysis (Berde, 1959; Harris, 1955; 1960; Kleiner & Cutler, 1963; Olivecrona, 1957). A variety of stimuli are known to bring about an oxytocin release. Suckling or mechanical stimulations of the mammary glands (Cross & Harris, 1952; Folley, 1947; Petersen, 1944), mechanical stimulation of uterus and vagina (Anderson, 1951; Harris, 1955; Harris & Pickles, 1953), distension of cervix and uterus during labour (Caldeyro-Barcia, Sica-Blanco, Alvarez, Pose, Poseiro, Méndez-Bauer, Fielitz, González-Panizza, Cabot, Branda, Escarcena, Coch & Brovetto, 1963; Cross, 1958) cause a reflex liberation of oxytocin. Thirst, an increase in osmotic pressure of the blood (Abrahams & Pickford, 1954; Anderson, 1951; Holland, Cross & Sawyer, 1959) and chemical agents such as actylcholine are also effective stimuli for oxytocin secretion (Abrahams & Pickford, 1954). It is also known that emotional stimuli augment (Abrahams & Pickford, 1954) or inhibit (Cross, 1955*b*) the milk ejection response.

Although these factors influencing oxytocin secretion from the neurohypophysis are assumed to initiate augmented activity in the hypothalamico-hypophysial system, no extensive study has been made of the relation between neuronal activities in the paraventricular nuclei and oxytocin release caused by the variety of neural and chemical influences mentioned. Electrical activity of single units of the paraventricular nucleus has been recorded in rabbits by Cross & Green (1959) and similar recordings have been made from pre-optic nuclei in the goldfish by Kandel (1964) but without simultaneous hormone assays.

In the work to be reported we recorded action potentials of neurones in the paraventricular nucleus and the related oxytocin output as assayed by the milk ejection response in post-partum cats. Thus, changes in electrical activity of hypothalamic neurones could be related to hormone secretion from the hypophysis. Our purpose was (1) to study neurone responses to osmotic, chemical and neural stimulation; (2) to cause reflex release of oxytocin and study the paraventricular neurone response under such circumstances.

METHODS

Normal female cats as well as post-partum cats, 2–3½ weeks after parturition, were anaesthetized with chloralose (35 mg/kg). The cranial bone of one side was removed to expose areas to be stimulated and to permit placement of recording electrodes. Leg nerves (gastrocnemius or tibial nerves) were exposed for stimulation in some cases, skin nerves were stimulated by inserting bipolar needle electrodes at the left side of the abdomen. The paramedian lobe of the cerebellum was stimulated through a bipolar electrode placed on the cortical surface. Excitations of mid-brain reticular formation at the level of the superior colliculus and of thalamic nuclei (ventrobasal complex) were accomplished by the use of concentric needle electrodes placed stereotaxically. The stimulating pulses used for excitation of central nervous structures were 5–15 V in intensity, 2 msec in duration and were given at a 50/sec frequency for 10–20 sec.

To stimulate nipples in post-partum cats, intermittent gentle suction was applied to one or more of the six or seven hypertrophied nipples found. Distension of the uterus was accomplished by inflating a rubber balloon, attached to the end of a catheter which had been inserted into the lumen of the uterus, by injection of 8–10 ml. of water.

For recording activity of single neurones in the paraventricular nucleus, steel microelectrodes were used. These were prepared as follows. Fine dental broaches were washed in acetone and their tips tapered down to 1–2 μ in size by electrolysis in a mixture of sulphuric and phosphoric acid (34 ml. concentrated sulphuric acid, 42 ml. ortho-phosphoric acid and 24 ml. of distilled water). After washing in water, then in alcohol, electrodes were insulated, except at their tips, by giving two coatings of Insl-X. Completeness of insulation at the tip of each electrode was tested by observing microscopically the formation of very small bubbles when a current was applied from a 1·5 V battery through a 250–500 kΩ resistance. The resistance of such electrodes was found to be approximately 300–500 kΩ. Electrical activities of neurones adjacent to the tip of such an electrode were monitored through condenser-coupled amplifiers (Tektronix-122) and were displayed on two oscilloscope screens. One was used to show the shape of action potentials and the other the continuous firing patterns.

Two hours before recording started, 60–100 ml. of water were introduced into the stomach to hydrate the animal. The head of the cat was firmly fixed in a stereotaxic holder and in many instances gallamine triethiodide (Flaxedil; 4 mg/kg) and artificial respiration were used to minimize body movement. The stereotaxic atlas of Snider & Niemer (1961) was followed in electrode orientation. In each preparation placements were limited to two positions 1 mm apart so that the histological tracing of recording sites could be done without confusion. By recording the depth of the electrode tip at each position along the track from which recordings were taken it was possible to determine whether or not units studied were within the paraventricular nucleus. This was done in the following manner. At the end of each impalement (electrode exploration) small amounts of iron were caused to be deposited at two sites in the track by passage of a 20 μA current for 30–45 sec. The brain was then perfused by way of the carotid arteries with 200 ml. of a mixture of potassium ferro- and ferricyanide solutions (3% solutions of each reagent mixed 1:1) which was followed by Ringer's solution. After fixation in formalin, frozen sections 40 μ in thickness were cut and stained with thionine. The point of iron deposit was identifiable as a small deep green spot. By marking two electrode positions, one at the top and the other at the bottom of the paraventricular nucleus region, the electrode tracks could be traced quite accurately in histological preparations (Fig. 1). Thus, it was possible to identify the positions from which neuronal discharges were recorded. In all experiments recordings from neurones outside the paraventricular nucleus were made as well as from within the nucleus to permit comparison of responses obtained.

For a close intra-arterial injection a small polyethylene tube was inserted deep into a

carotid artery without stopping the circulation. Special care was taken to introduce solutions at body temperature. One millilitre of 1 % Evans-blue solution injected into a carotid artery in this manner appeared in the ipsilateral retinal artery in 3–5 sec after the injection. This was interpreted to indicate that any solution thus injected into the carotid artery will reach the hypothalamus in a similar short period of time (Koizumi, Ishikawa & Brooks, 1964; Verney, 1947).

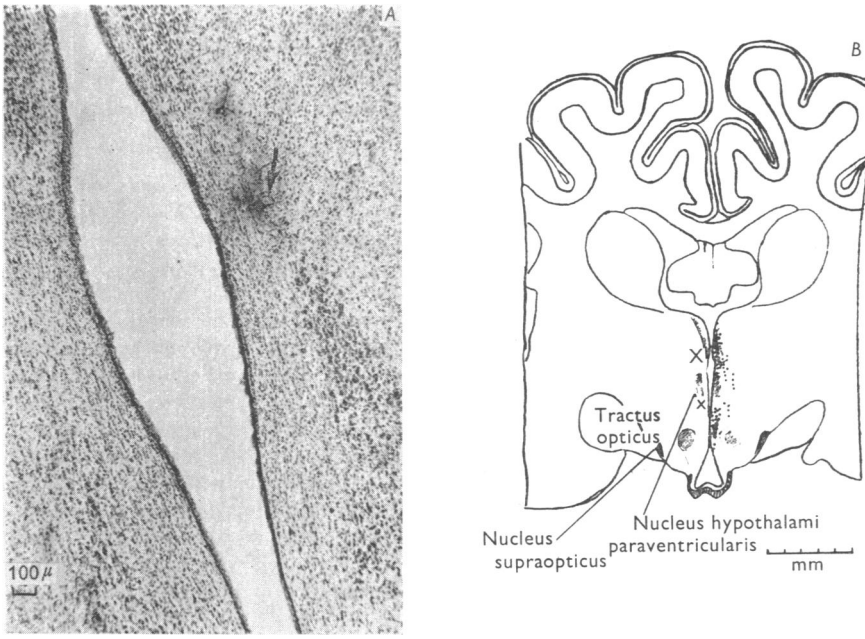

Fig. 1. *A*. Photomicrograph of paraventricular nucleus region showing the iron deposit from a recording electrode tip (↓). *B*. Tracing of entire histological preparation including area shown in *A*. The left half shows two markings (X) along the electrode track made when tip lay below and then above paraventricular nucleus. Right half shows positions of all 'extra-paraventricular' neurones from which recordings were made (.). Histological preparations containing neurone markings were projected upon the tracing in making this illustration. Slight differences in frontal planes of histological preparations were adjusted so that the relation between recording sites and paraventricular nucleus could be correctly shown.

Post-partum cats were used in experiments involving measurement of milk ejection to permit assay of oxytocin release. Kittens were separated from the mother cat the night before the experiment to ensure full mammary glands. A single mammary duct was cannulated with a fine nylon tube drawn to a desired size; polyethylene tubing was found not to be stiff enough for easy cannulation of the duct. After insertion the nylon catheter was fixed to the skin by adhesive tape and connected to polyethylene tubing which was increased in diameter by several steps and finally attached to a transducer. The whole system was filled with heparin-Ringer's solution. Milk ejection from a single mammary duct was measured as a change in pressure (mm H_2O) recorded by a Statham transducer and a Gilson 4-channel recorder. After cannulation, 5 or 10 m-u. oxytocin (Pitocin, Parke Davis Co.) was injected into a radial vein to initiate a milk ejection response. This usually served as a

test for adequacy of the recording system and also provided means for quantitating the oxytocin release during the experiment. The increase in milk ejection pressure produced by 5 m-u. of oxytocin varied greatly depending on the condition of the mother cat and the number of days after parturition. Responses obtained from different nipples or ducts also varied. There are several ducts in one nipple and a change in duct cannulated in the same nipple or a change to another nipple was sometimes necessary during an experiment in order to obtain a satisfactory response. Calibration by oxytocin injection was repeated after each experimental procedure since the milk ejection response produced by oxytocin sometimes changed during the long period of experimentation (6–7 hr). Blood pressure from a femoral artery was recorded continuously.

RESULTS

Responses to osmotic and chemical stimulations

Neurones of the paraventricular nucleus showed some activity in the 'resting' condition. The rate of discharge was approximately 2–6/sec, occasionally less than 1 and rarely exceeding 10/sec frequency.

The sensitivity of neurones to changes in the osmotic pressure of blood was estimated by injections of hypertonic saline into a carotid artery (Jewell & Verney, 1957; Verney, 1947). If neurones showed a definite increase in rate of firing for more than 2–3 min, following a 10–15 sec duration intra-arterial injection of 1 ml. of 1 M-sodium chloride solution, they were considered to be 'osmosensitive' (Koizumi *et al.* 1964). According to Verney's calculations (Verney, 1947) this injection should have caused a 63 % increase in carotid blood osmotic pressure and a discharge of 0·5 m-u. posterior pituitary hormone. Seventy-four per cent of the paraventricular nucleus neurones tested were found to belong to this category in normal cats. The locus of these units was confirmed histologically. Increases in firing rate of these neurones, however, did not reach as high values (200–300 % of control rate) as those observed to occur in the supraoptic nucleus (Koizumi *et al.* 1964), even when the volume and the concentration of sodium chloride solution injected was greater. Figure 2 shows the average response of 49 'osmosensitive' neurones of the paraventricular nucleus. Twenty-three per cent of all cells studied were not influenced by this stimulation and 3 %, i.e. only two neurones, showed a decreased rate of discharge.

In post-partum cats osmosensitive cells showed a similar response. In these animals, however, the augmented cellular discharges were accompanied by a mild increase in milk ejection pressure (Fig. 3). Comparison of reactions of normal and post-partum females showed that a large number of neurones in the paraventricular nucleus of the post-partum cat (17 % of total) were depressed by osmotic stimulation; 25 % of the cells were unaffected, thus 58 % of the neurones were 'osmosensitive'. A decreased rate of discharge following injection of hypertonic saline solution

occurred only when a 2 M-NaCl solution was injected in attempting to obtain a good milk ejection response from post-partum cats. Depression, therefore, was probably due to discharges of sensory afferents in or near the blood vessels or to a direct effect on the hypothalamic neurones rather than to osmotic changes in the blood. No such inhibition was recorded when hypertonic saline of lower concentration was administered by slow

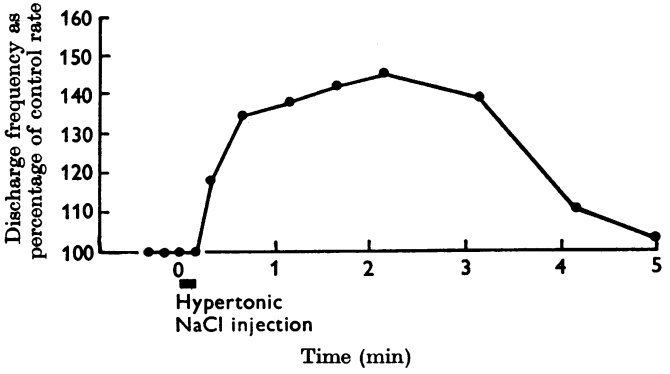

Fig. 2. Average intensity and course of response in forty-nine neurones of the paraventricular nucleus to intra-arterial injection of hypertonic saline solution. (1 ml. of 1 M-NaCl.) Time of injection is shown by a black bar.

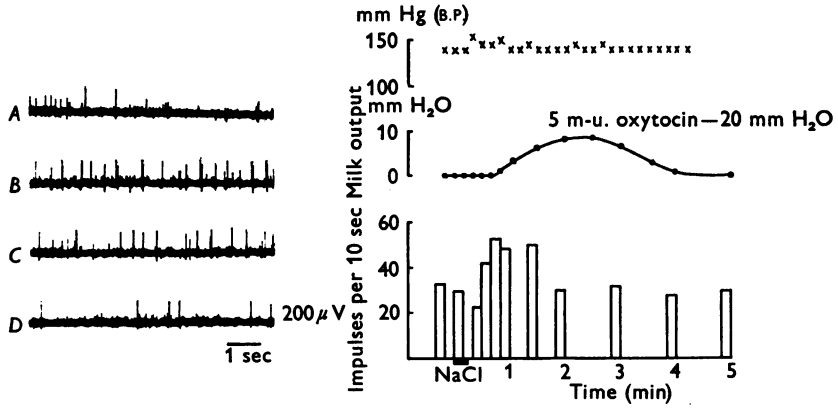

Fig. 3. Effects of osmotic stimulation on paraventricular neurone discharges, on milk ejection pressure, and on blood pressure. Time of intracarotid injection of 1 ml. of 1 M-NaCl is marked by a bar in the lower right-hand graph. The number of impulses in paraventricular nucleus neurones during 10 sec intervals before and after injection is shown in the bar graph. The corresponding time of milk ejection response, recorded as a mammary duct pressure, is shown in the right middle tracing. A calibrating dose of 5 m-u. oxytocin given intravenously resulted in a pressure change of 20 mm H_2O. Blood pressure is shown in the top right-hand tracing. Actual records of neurone discharges are given at the left: *A*, control period; *B*, 30 sec after injection; *C*, 2 min after; and *D*, 5 min after osmotic stimulation. Spikes retouched.

infusion. Cross & Green (1959) also observed the depression in activity of paraventricular nucleus neurones following strong osmotic stimulation. It was interesting to note that some neurones recovered from this early depression in the first 1 or 2 min after injection; their activity was then augmented and milk ejection pressure began to rise.

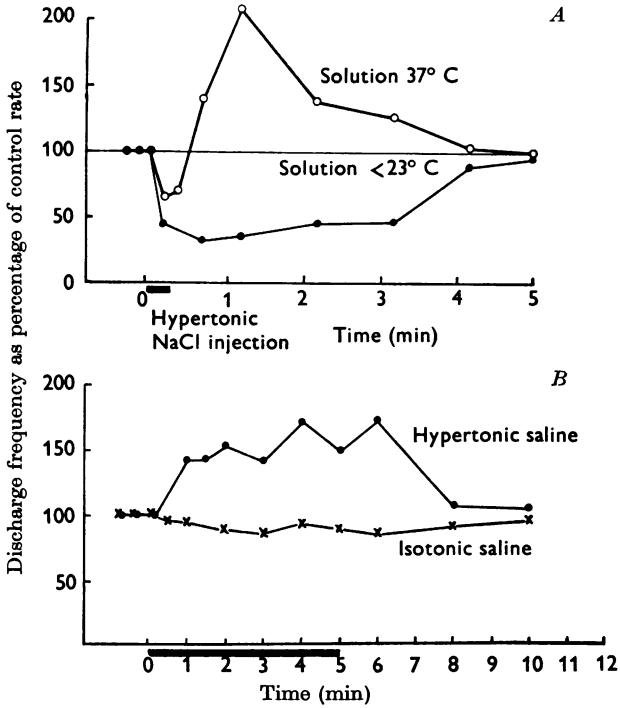

Fig. 4. *A*. Comparison of responses of the same paraventricular nucleus neurone to hypertonic saline (1 ml. of 1 M-NaCl) injected into a carotid artery when solution was at different temperatures. *B*. Effects of long lasting infusion of hypertonic NaCl solution (0·93 M) and isotonic saline on the same neurone. Infusion lasted for 5 min at a rate of 0·5 ml./min.

Another important finding was that when the temperature of the solution injected into the carotid artery was subnormal, a depression of neurone activity frequently took place. This was confirmed by introducing hypertonic solution at 37° C and then at room temperature (26° C) into the carotid artery and recording cellular response from the same neurone. The latter injections produced a definite depression of the response, while the warm solution caused an augmentation (Fig. 4*A*). This depression, however, did not occur every time a cool solution was injected. Hypertonic saline of high concentration though administered at body temperature also occasionally caused temporary depression of neurone activity. Inhibition,

therefore, can result from more than one type of stimulation and must involve something other than a response to changes in osmotic pressure.

In a few experiments hypertonic solution at low concentration (0·93 M-NaCl) was administered intra-arterially by an infusion at a rate of 0·5 ml. per minute for 4–8 min. This was done to eliminate sensory discharges from blood vessel walls and to provide long-lasting osmotic changes of small magnitude (approximately 5 % increase) in order to produce situations somewhat similar to those occurring physiologically (Verney, 1947). Some forty-six of the paraventricular nucleus were thus tested and 57 % of these neurones were found to be definitely excited by such an osmotic stimulation (Fig. 4*B*). We did not observe a single cell showing a reduced rate of firing after the infusion began.

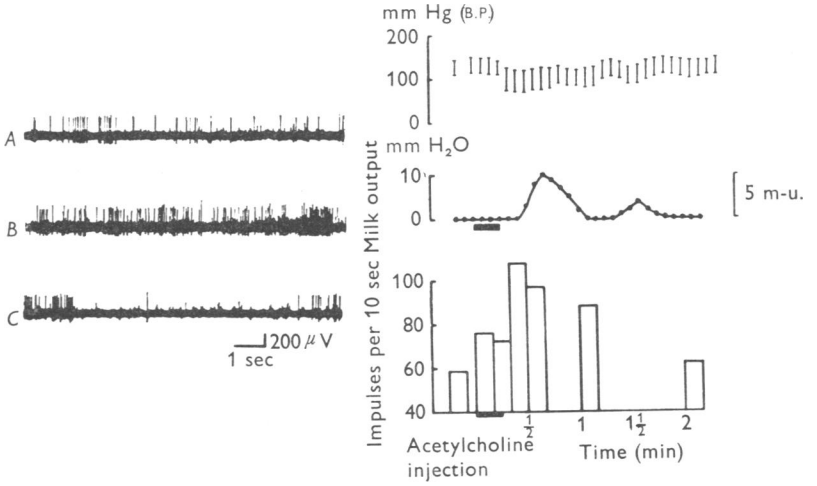

Fig. 5. Effect of acetylcholine on neurone activity in paraventricular nucleus, on milk ejection response and on blood pressure. Acetylcholine chloride, 40 μg, was given intra-arterially in 15 sec (—). Cellular discharges are shown on the left. *A*, control; *B*, 20 sec after the injection; and *C*, 5 min after the injection. The rates of discharge are plotted in the bar graph at the bottom right. Mammary duct pressure is shown at centre on right. The calibration sign designates maximum pressure change caused by 5 m-u. of oxytocin given intravenously. Blood pressures recorded from femoral artery are shown at top right. Spikes retouched.

A comparison was made between reactions of neurones definitely located in the paraventricular nuclei and adjacent cells. The majority of these peripheral cells showed no response to injections of 1 M-NaCl but some 33 % were 'osmosensitive'. Though these cells lay outside the boundaries of the paraventricular nuclei as usually defined by histological criteria (Fig. 1*B*) it is very likely that they serve the same function as do the osmosensitive neurones concentrated in the nuclei.

Neurones in the paraventricular nucleus were sensitive to acetylcholine

as were those of the supraoptic nuclei. Acetylcholine chloride (40–80 μg) injected into the carotid artery excited these neurones as indicated by an increase in rate of discharge. As shown in Fig. 5, this was accompanied by a milk ejection response equivalent to that produced by 5 m-u. of oxytocin. All neurones in the paraventricular nucleus which were reflexly excited by nipple or uterus stimulation, as discussed below, were sensitive to acetylcholine.

Responses to excitation of central and peripheral afferents

Neurones in the paraventricular nucleus were not much affected by electrical stimulations of afferent nerves nor of central nervous structures. Stimulations of skin or afferent nerves from leg muscles, such as tibialis and gastrocnemius, produced augmentation of the rate of discharges in

TABLE 1. Percentages of different categories of responses from cells of the paraventricular nucleus and from cells in adjacent tissue (Fig. 1*B*) elicited by various stimuli

		Paraventricular nucleus neurones								Non-paraventricular nucleus neurones			
		Normal				Post-partum				Normal			
		Response											
	Stimuli	Augmented(%)	Depressed (%)	Unaffected (%)	No. of cells tested	Augmented (%)	Depressed (%)	Unaffected (%)	No. of cells tested	Augmented (%)	Depressed (%)	Unaffected (%)	No. of cells tested
NaCl	Quick injection	74	3	23	67	58	17	25	48	33	7	60	60
	Infusion	57	0	43	46	—	—	—	—	39	0	70	24
Acetylcholine		—	—	—	—	100	0	0	21	—	—	—	—
Nipple:	Suction	—	—	—	—	83	0	17	48	—	—	—	—
	Electrical	32	41	27	62	45	10	45	27	23	44	33	66
Uterus		—	—	—	—	84	0	16	46	—	—	—	—
Skin		48	32	20	65	56	16	28	60	38	25	37	67
Cerebellum		38	24	38	61	25	25	50	28	33	40	27	66

some paraventricular nucleus cells and depressed others, but one third of the population was unaffected. The magnitude of changes evoked by such stimulations was not as marked as that observed in supraoptic nucleus cells (Koizumi *et al.* 1964). Electrical stimulation of central structures, cerebellum, mid-brain reticular formation, thalamus, also gave similar results, augmenting the discharge rate in some, depressing it in others and not influencing it in many. Results of these stimulations were not considered to indicate specific control since neurones outside the paraventricular nucleus region showed similar response (Table 1). When the same stimula-

tions were performed in post-partum cats, results were quite similar. In most instances no increase in milk ejection pressure was detected, but there were a few experiments in which it did occur.

Results such as these indicate that neurones of the paraventricular nucleus can be influenced by incoming sensory inputs if these are strong enough. Such reactions, however, might better be thought of as part of a general body response rather than as responses normally controlled by skin or muscle afferents or by these higher centres which were stimulated.

Reflex excitation of paraventricular nucleus neurones

Although paraventricular nucleus neurones were not specifically sensitive to the above-mentioned stimuli, they were easily excited by stimulations of the nipples by suction or by stimulation of the uterus by distension, particularly in post-partum cats. Electrical stimulation of the nipples failed to excite neurones (cf. Cross & Green, 1959), but gentle intermittent suction applied from a vacuum pump for a prolonged period of time

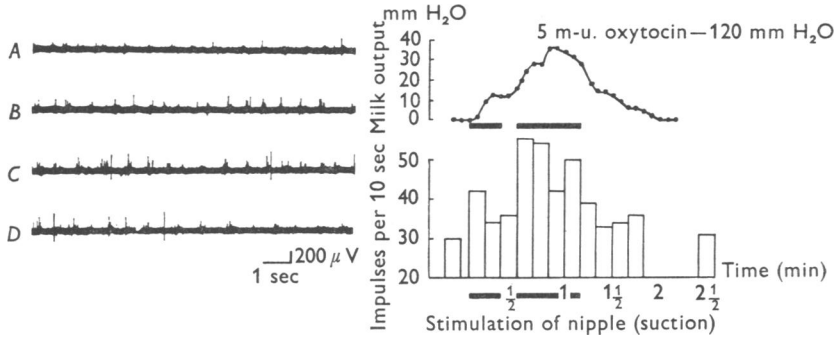

Fig. 6. Effects of suction stimulations of nipple (—) on paraventricular nucleus neurone activity in 10 sec intervals (bar graph) and mammary duct pressure (upper right). Left: cellular discharge; *A*, control; *B* and *C*, during; and *D*, 3 min after suction stimulation of nipple. Duct pressure from subsequent injection of oxytocin shown.

(1 min) increased the neurone discharge and elicited a milk ejection response. Figure 6 shows the relation between augmented discharges of a paraventricular neurone and the increase in milk ejection pressure. Distension of the uterus produced a similar effect (Fig. 7). The frequency of discharge of impulses from the paraventricular nucleus neurones was more than doubled by this uterine stimulus and assays showed that oxytocin discharge attained a 3 m-u. equivalence. Systemic blood pressure was not changed much by these procedures and it is reasonable to assume that milk output was caused by oxytocin release resulting from an increased activity in the paraventricular nucleus.

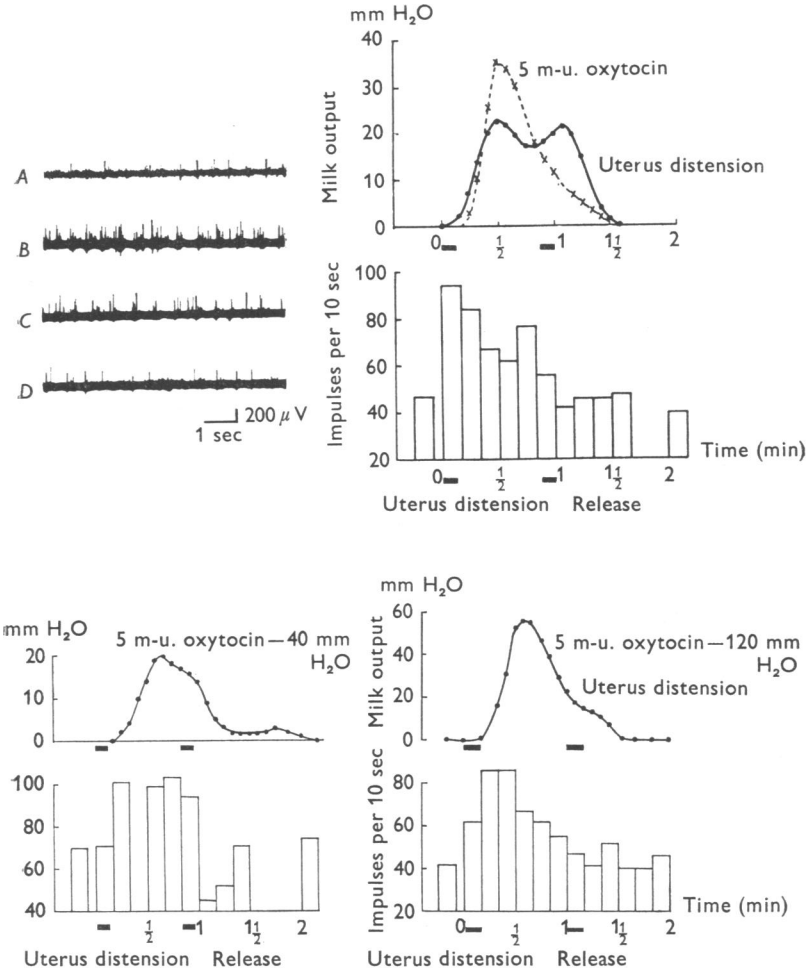

Fig. 7. Responses similar to those shown in Fig. 6, but caused by distension of uterus. Bars (— —) indicate the time at which a balloon inside the uterine cavity was inflated by introductions of 7 ml. of water and then deflated. Lower bar graph of top records shows neurone discharges during 10 sec intervals before, during and after distension. Duct pressures generated by distension and by injection of 5 m-u. oxytocin during a similar period (upper right). At left: unit discharges; *A*, control; *B* and *C*, during; and *D*, after the uterus distension. Lower two units of illustration show other examples of the effects of uterus distension. Left and right-hand figures from two other preparations. Pressures developed from 5 m-u. oxytocin indicated.

Other changes produced in the paraventricular nucleus

When unit discharges were recorded from paraventricular nucleus neurones and the mammary duct was cannulated, we sometimes observed gradual changes in mammary duct pressure without any external stimulus

or obvious change of the animals' condition. This seemingly 'spontaneous' change was accompanied by or preceded by an increased rate of discharge of neurones in the paraventricular nucleus. It was not possible to trace the exact cause of this change but the phenomenon showed that oxytocin release could be nicely correlated with neuronal activity in the hypothalamus.

When an electrode recording activity was moved to another position or when a current was passed through the electrode to mark its position, a slight increase in mammary duct pressure was generally registered. This must have been caused by a direct mechanical or electrical stimulation of paraventricular nucleus cells. Repetitive electrical pulses applied to this nucleus through a bipolar stimulating electrode, on the other hand, failed to produce any milk output in our experiment. This may have been due to the simultaneous production of sympathicoadrenal discharges which inhibit milk ejection at the periphery (Cross, 1953; 1955*a*; Cross & Harris, 1952). Movement of a fine tipped recording electrode or passage of a small current through this tip, however, may produce a very localized change without evoking sympathetic discharge.

Stimulation of the pituitary stalk caused some milk ejection response in cats as in rabbits (Cross & Harris, 1952). The amount recorded in our experiment was rather small compared to that previously reported. We used hemispherectomized preparations in order to visualize the pituitary stalk and this may have interfered in part with circulation to the pituitary gland. A high percentage of descending fibres may also have been inactivated.

DISCUSSION

Paraventricular and supraoptic nuclei of the hypothalamus control the production and release of posterior pituitary hormones, ADH (vasopressin) and oxytocin. The problem of the role played by each nuclear system in controlling two types of hormone or a hormone having two functions is still unsettled. Extracts of the supraoptic nucleus, like extracts of the paraventricular nucleus, possess both oxytocic and pressor-antidiuretic properties (Van Dyke, Adamsons & Engel, 1957). In most animals vasopressin (ADH) is always present in higher concentration than oxytocin in the hypothalamus, and the neurohypophysis. However, the ratio of vasopressin content to oxytocin varies greatly from one part of the neurosecretory system to another and from one species to the next (Berde, 1959). The vasopressin oxytocin ratio in supraoptic nuclei is fifteen in dogs, three in sheep, but in paraventricular nuclei it is 9 and 0·7 respectively (Lederis, 1962). This relation does not hold, however, with respect to the hormone release caused by stimulations.

Electrical stimulation of the supraoptico-hypophysial tract in rabbits

evokes liberation of a secretion which has more oxytocic activity than antidiuretic-pressor activity; a ratio of approximately 4:1 prevails. Physiological stimuli such as blood osmotic pressure changes, emotional disturbances, suckling, or coitus were found to cause release of both hormones from the pituitary gland, but in different ratios. It has been reported that the oxytocic-pressor (antidiuretic) activity ratio of posterior pituitary hormone released by intracarotid hypertonic saline injection is 30:1, and by suckling 100:1 (Harris, 1960). These complicated data at least suggest the possibility of a selective activation of hypothalamic neurones which may favour the release of one hormone or the other. There is evidence which shows that bilateral destruction of the paraventricular nuclei causes loss of oxytocic material from the neurohypophysis without affecting the antidiuretic-pressor principle (Olivecrona, 1957). Others have suggested the existence of two different systems in the brain responsible for control of the two hormones (Rothballer, 1966).

Our studies on electrical activities of supraoptic and paraventricular nuclei have shown that, although the pattern and frequency of neurone discharges in the two nuclei are very similar, changes of activity recorded from the two differ considerably under varying conditions. Neurones of the supraoptic nucleus are more sensitive than those of the paraventricular nucleus to changes in concentrations of osmotically active materials in the blood. They are also more under the control of excitatory and inhibitory influences from many central nervous structures such as the limbic system, motor cortex, cerebellum (Koizumi *et al.* 1964; Suda, Koizumi & Brooks, 1963) as well as from the reticular formation, thalamus, hypothalamus (Ishikawa, Koizumi & Brooks, 1965). Paraventricular nucleus neurones, on the other hand, are less sensitive to these stimuli but respond readily to reflex excitation caused by nerve impulses arising from nipples, vagina and uterus in the post-partum state. Acetylcholine seems to excite both varieties of neurone to the same degree. Thus, it seems clear that cells in paraventricular and supraoptic nuclei have their own characteristic way of responding to a variety of stimuli. When neurones in the paraventricular nuclei are selectively excited by certain impulses, a release of oxytocic hormone predominates. Another stimulus may excite neurones in supraoptic nuclei more than those in the paraventricular nuclei thus causing release of a larger amount of antidiuretic-pressor hormone than oxytocin. The problem still remains, however, why more oxytocin is liberated by any type of stimulus, while the neurohypophysis and hypothalamus generally contain more ADH or vasopressin than oxytocin. More studies of this type are certainly needed to clarify the problem.

A considerable number of neurones, adjacent to but outside the cell grouping identified as the paraventricular nucleus, behaved as did cells of

that nucleus. It is reasonable to assume that cells of similar property and function are somewhat diffusely scattered and not all tightly concentrated in one circumscribed area. Similarly, it seems reasonable that all units are not equally affected by peripheral receptors. Cells of the paraventricular nuclei are influenced by fewer afferents than are the neurones of the supraoptic nucleus, but this is a relative matter. The fact that in a few preparations stimulation of skin nerves evoked milk ejection need not be interpreted as contrary to the general conclusion that control of the paraventricular nucleus cells chiefly arises from the mammary glands and uterus.

It is always difficult to prove that electrical activity recorded from cells of the paraventricular nuclei is the direct cause of oxytocin release which in turn produces milk ejection response. In our work, changes of frequency of discharges of hypothalamic neurones were very closely related to changes in mammary duct pressure under the various conditions created by reflex or chemical excitation. We also recorded action potentials from the pituitary stalk under similar conditions; they followed the changes occurring in the paraventricular nuclei and could be used to predict responses of the mammary ducts (Ishikawa, Koizumi & Brooks 1965). Wherever the site of production of oxytocin may be, it has been firmly established that the paraventricular nucleus does control oxytocin release from the pituitary gland and that this is the direct cause of the milk ejection response. Thus, it is reasonable to assume that the neurones in the paraventricular nucleus, from which we were able to record action potentials, are chiefly responsible for oxytocin release, although some contribution from the supraoptic nucleus is not denied.

This investigation was supported principally by a grant from USPHS (NB-00847 (09)) and partly from USPHS (5 R 01 HD 00102–01).

REFERENCES

ABRAHAMS, V. C. & PICKFORD, M. (1954). Simultaneous observations on the rate of urine flow and spontaneous uterine movements in the dog, and their relationship to posterior lobe activity. *J. Physiol.* **126**, 329–346.

ANDERSON, B. (1951). Some observations on the neurohormonal regulation of milk ejection. *Acta physiol. scand.* **23**, 1–7.

BERDE, B. (1959). *Recent Progress in Oxytocin Research.* Springfield, Ill.: Charles C. Thomas.

CALDEYRO-BARCIA, R., SICA-BLANCO, Y., ALVAREZ, H., POSE, S. V., POSEIRO, J. J., MÉNDEZ-BAUER, C., FIELITZ, C., GONZÁLEZ-PANIZZA, V. H., CABOT, H. M., BRANDA, L., ESCARCENA, L., COCH, J. A. & BROVETTO, J. (1963). Oxytocin in labor and in milk ejection. In *Perspectives in Biology*, ed. CORI, C. F., pp. 62–74. Amsterdam: Elsevier Publishing Co.

CROSS, B. A. (1953). Sympathetico-adrenal inhibition of the neurohypophysial milk-ejection mechanism. *J. Endocrin.* **9**, 7–18.

CROSS, B. A. (1955*a*). The hypothalamus and the mechanism of sympathetico-adrenal inhibition of milk ejection. *J. Endocrin.* **12**, 15–28.

CROSS, B. A. (1955*b*). Neurohormonal mechanisms in emotional inhibition of milk ejection. *J. Endocrin.* **12**, 29–37.

CROSS, B. A. (1958). On the mechanism of labour in the rabbit. *J. Endocrin.* **16**, 261–276.

CROSS, B. A. & GREEN, J. D. (1959). Activity of single neurones in the hypothalamus. Effect of osmotic and other stimuli. *J. Physiol.* **148**, 554–569.

CROSS, B. A. & HARRIS, G. W. (1952). The role of the neurohypophysis in the milk-ejection reflex. *J. Endocrin.* **8**, 148–161.

VAN DYKE, H. B., ADAMSONS, K., JR. & ENGEL, S. L. (1957). The storage and liberation of neurohypophysial hormones. In *The Neurohypophysis*, ed. HELLER, H., pp. 65–73. London: Butterworth Scientific Publ.

FOLLEY, S. J. (1947). Endocrine control of the mammary gland. II. Lactation. *Br. med. Bull.* **5**, 142–148.

HARRIS, G. W. (1955). *Neural Control of Pituitary Gland.* London: Edward Arnold, Ltd.

HARRIS, G. W. (1960). Central control of pituitary secretion. In *Handbook of Physiology, Neurophysiology*, sect. 1, vol. II, chapt. 39, pp. 1007–1038. Washington, D.C.: Amer. Physiol. Soc.

HARRIS, G. W. & PICKLES, V. R. (1953). Reflex stimulation of the neurohypophysis (posterior pituitary gland) and the nature of posterior pituitary hormone(s). *Nature, Lond.*, **172**, 1049.

HOLLAND, R. C., CROSS, B. A. & SAWYER, C. H. (1959). Effects of intracarotid injections of hypertonic solutions on the neurohypophysial milk ejection mechanism. *Am. J. Physiol.* **196**, 791–795.

ISHIKAWA, T. KOIZUMI, K. & BROOKS, C. McC. (1965). Electrical activity recorded from the pituitary stalk of the cat. *Am. J. Physiol.* (In the Press.)

ISHIKAWA, T., KOIZUMI, K. & BROOKS, C. McC. (1965). Activity of supraoptic nucleus neurons of the hypothalamus. *Neurology.* (In the Press.)

JEWELL, P. A. & VERNEY, E. B. (1957). An experimental attempt to determine the site of the neurohypophysial osmoreceptors in the dog. *Phil. Trans. R. Soc.* **240**, 197–324.

KANDEL, E. R. (1964). Electrical properties of hypothalamic neuroendocrine cells. *J. gen. Physiol.* **47**, 691–717.

KLEINER, C. R. & CUTLER, R. E. (1963). The neurohypophysis. *A. Rev. Physiol.* **25**, 385–432.

KOIZUMI, K., ISHIKAWA, T. & BROOKS, C. McC. (1964). Control of activity of neurons in the supraoptic nucleus. *J. Neurophysiol.* **27**, 878–892.

LEDERIS, K. (1962). The distribution of vasopressin and oxytocin in hypothalamic nuclei. In '*Neurosecretion*'. *Mem. Soc. Endocr.* **12**, 227–236.

OLIVECRONA, H. (1957). Paraventricular nucleus and pituitary gland. *Acta physiol. scand.* **40**, Suppl. 136.

PETERSEN, W. E. (1944). Lactation. *Physiol. Rev.* **24**, 340–371.

ROTHBALLER, A. (1966). Pathways of secretion and regulation of posterior pituitary factor. *Res. Publ Ass. Res. nerv. ment. Dis.* **43** (In the Press.)

SNIDER, R. S. & NIEMER, W. T. (1961). *A Stereotaxic Atlas of the Cat Brain.* Chicago: University of Chicago.

SUDA, I., KOIZUMI, K. & BROOKS, C. McC. (1963). Study of unitary activity in the supraoptic nucleus of the hypothalamus. *Jap. J. Physiol.* **13**, 374–384.

VERNEY, E. B. (1947). The antidiuretic hormone and the factors which determine its release. *Proc. R. Soc.* B, **135**, 25–106.