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RESPONSES OF SUPRAOPTIC NEURONES IN THE INTACT AND DEAFFERENTED RAT HYPOTHALAMUS TO INJECTIONS OF HYPERTONIC SODIUM CHLORIDE

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SUMMARY

1. Recordings were made from a total of fifty-three neurones in the supraoptic nuclei of four groups of rats: intact rats, animals in which the hypothalamus had been partly denervated by anteriorly or posteriorly placed semicircular cuts, and rats with a totally deafferented hypothalamus.

2. When first encountered, cells from intact animals fired at a mean rate of $5{\cdot}08 \pm 0{\cdot}78$ spikes/sec, those from posteriorly isolated hypothalami at $3{\cdot}93 \pm 0{\cdot}63$ spikes/sec, those from the anteriorly isolated hypothalami at $2{\cdot}05 \pm 0{\cdot}83$ spikes/sec, and those from totally isolated hypothalami at $0{\cdot}99 \pm 0{\cdot}46$ spikes/sec.

3. When stimulated osmotically by an intraperitoneal injection of 1 ml. 1·5 M-NaCl, eight out of eight cells in intact rats showed a significant increase in firing rate between 20 and 30 min after the injection. Six out of nine cells in posteriorly isolated hypothalami showed significant but smaller responses. No increase in firing rate could be detected in seven cells from totally isolated hypothalami or from eight cells in hypothalami partly isolated by anterior cuts.

4. The results imply that under the conditions of these experiments the spontaneous activity of the supraoptic nucleus in intact animals was maintained by an extrahypothalamic excitatory input, that partial hypothalamic isolation reduced its intensity, possibly by unmasking an inhibitory input, and that total isolation reduced it to an even greater extent. Osmotic activation of supraoptic cells was only possible when the anterior connexions of the hypothalamus were intact. Thus the cerebral osmoreceptors for vasopressin release may be situated outside the supraoptic nuclei.

INTRODUCTION

Jewell & Verney (1957) infused hypertonic solutions into the common carotid artery to release ADH and occluded branches of the artery to restrict the distribution of the infusions. They concluded that the cerebral osmoreceptors mediating ADH release, in the dog, must be in or near the supraoptic nucleus. Later, Sundsten &

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Sawyer (1961) in the rabbit, and Woods, Bard & Bleier (1966) in the cat and rat, found that to maintain water balance and responses to diuretic and antidiuretic stimuli the pituitary needed to be connected to only a small island (about 30%) of hypothalamic tissue adjacent to the optic chiasma. However, in neither of these studies was either the unit activity of the supraoptic nuclei or the plasma vasopressin concentration determined. More recently Sladek & Knigge (1977*a*) showed that the vasopressin output of the neurohypophysial system in organ culture increased with increased osmotic pressure of the medium and Leng (1979) showed that application of hypertonic NaCl solution to neurones of the supraoptic nucleus by a microtap method excited such neurones. Peck & Blass (1975) on the other hand, having made microinjections of hypertonic solutions into different forebrain regions of the rat, concluded that the neurosecretory cells in the supraoptic nucleus did not themselves seem to be osmoreceptors. Moreover, osmoresponsive neural structures have been described in the medulla (Clemente, Sutin & Silverstone, 1957) mid-brain (Malmo, 1976), preoptic area (Weiss & Almli, 1975), amygdala, hippocampus and olfactory bulb (Sawyer & Gernandt, 1956).

In an attempt to resolve the mechanism underlying these apparently conflicting results, we have re-investigated the site of the cerebral osmoreceptors by recording from single identified neurones in the supraoptic nucleus of the partly or totally deafferented hypothalamus during intraperitoneal injections of hypertonic NaCl solution. Such injections increase plasma osmotic pressure and increase the firing rate of neurones in the supraoptic nucleus, and their effects can be mimicked by hypertonic injections of LiCl or mannitol. They therefore probably act by increasing plasma osmotic pressure (Brimble, Dyball & Forsling, 1978). A preliminary report of this work has already been published (Dyball & Prilusky, 1980).

METHODS

Animals

Nineteen male Wistar rats, 250–350 g in body weight, were used in the investigation. They were maintained at 21 °C with 10 hr dark and 14 hr light periods and given food (Diet 41B, Grain Harvesters) and water *ad libitum*.

Surgical preparation

On the day of the experiment the rats were anaesthetized by a single intraperitoneal injection of urethane (ethyl carbamate; 1·25 g/kg). If additional anaesthesia was required during surgery, small doses (5 mg) of methohexitane sodium (Brietal, Elanco) were given intraperitoneally. A flexible cannula (Silastic, Dow Corning, i.d. 0·02 in., o.d. 0·037 in.) was then inserted into the right atrium through the right jugular vein to collect blood samples (0·6 ml.) for plasma osmotic pressure determination (carried out by the freezing-point method using an Advanced Osmometer). An addition cannula (Portex PP 25) was inserted into the right saphenous artery to monitor blood pressure.

The animals were then fixed in a stereotaxic frame and a single large burr hole, extending some 5 mm along the mid-line, was drilled through the skull. After ligation of the sagittal sinus the dura was incised and the brain exposed for the introduction of the knife used to make the islands and the placement of recording and stimulating electrodes.

Hypothalamic deafferentation

Using a modification of the method of Halasz & Pupp (1965), three different types of hypothalamic lesion were made, anterior semicircular cuts, posterior semicircular cuts and total hypothalamic deafferentation (see Figs. 1 and 3).

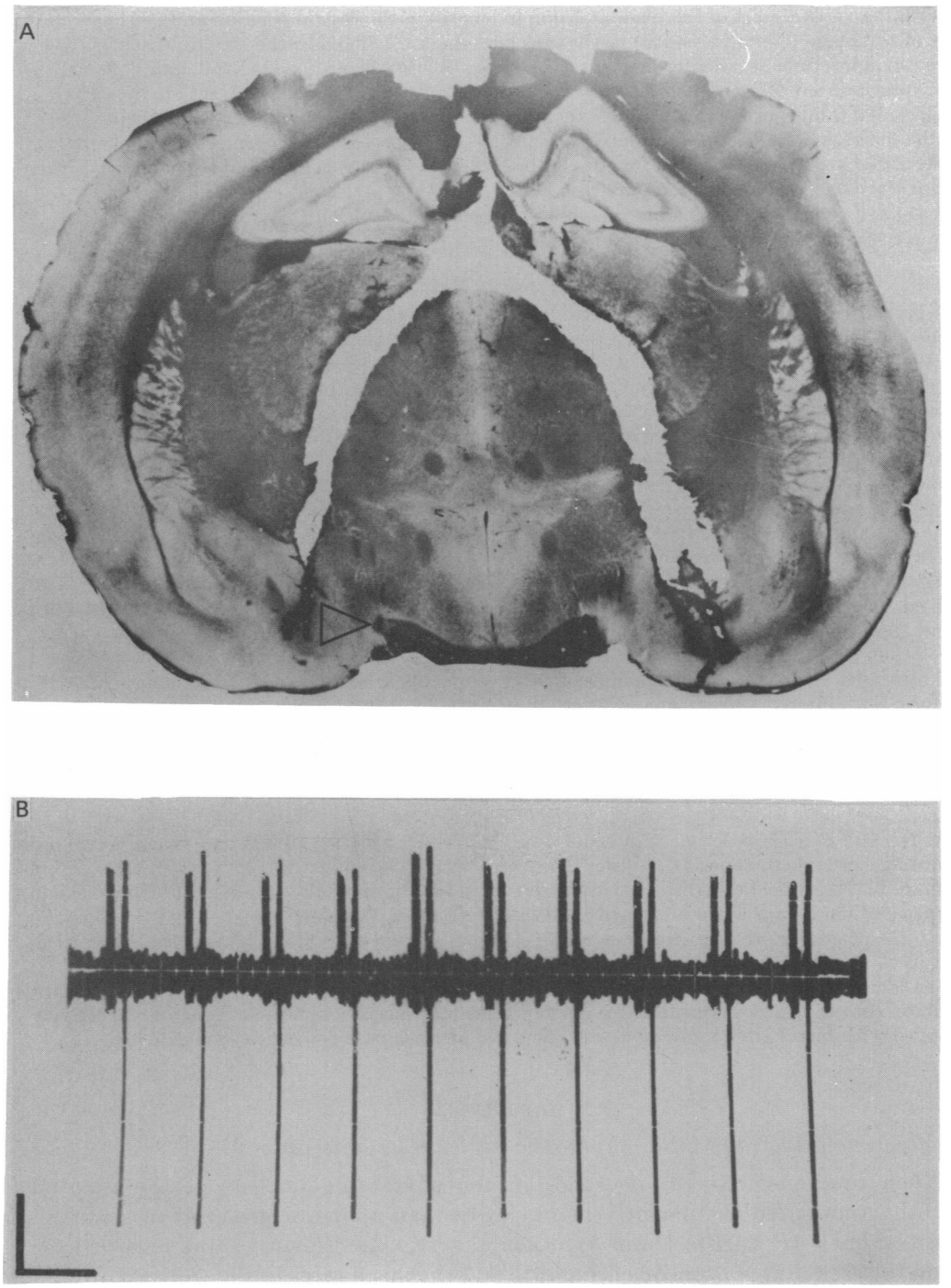

Fig. 1. *A*, a photomicrograph of the frontal section of the rat brain to illustrate the size of the hypothalamic island from which recordings were made. A Prussian blue spot can be seen just to the left of the optic tract on the left side (arrow). *B*, single sweep of oscilloscope trace to show that regular antidromic spikes (biphasic deflexions) occurred during a period of continuous neural stalk stimulation (small upwards deflexions) at 10 Hz and that spike height did not substantially alter (scale mark 100 msec and 0·1 mV).

With the vertical part of the blade (4·7 mm in length) in the rostral position and the horizontal part of the blade (2 mm in length) in the mid line, the knife (fixed in the electrode holder of the stereotaxic instrument) was lowered into the brain. When the tip touched the cranial floor, the knife was rotated 90° on each side so as to produce a half-dome shaped cut in the anterior deafferented group; or a complete 360° rotation in the totally deafferented hypothalamus group. In the posterior deafferented group the same general procedure described for the anterior deafferented group was followed, except that the vertical blade was placed in a posterior position before starting the surgical procedure (see Figs. 1 and 3).

A control group of experiments was also performed in which all the surgical procedures were followed except that the knife was not rotated.

Electrophysiological recording

A bipolar concentric stimulating electrode (NE 100, Rhodes Medical Instruments) was lowered in the mid line at an angle of 13° from the vertical with the lower end forward, so that its tip rested on the neural stalk (Brimble & Dyball, 1977). InslX (InslX Products Corp.) coated steel micro-electrodes (approx. tip diameter 2 μm) were then lowered into the supraoptic nucleus, and extracellular action potentials recorded using conventional techniques. When a unit was encountered which could be antidromically activated by stimulation of the neural stalk (single biphasic pulse, 1 mA peak-to-peak, of 2 msec duration), two blood samples of 0·6 ml. were taken through the atrial cannula for plasma osmotic pressure determination. After each sample had been taken, blood volume was immediately restored by the injection of 0·6 ml. isotonic NaCl solution through the same cannula. The blood sampling technique itself had only a transient (less than 2 min) effect on the firing rate of the cells tested.

After at least 20 min of recording, the osmotic pressure was altered by an intraperitoneal injection of 1 ml. 1·5 M-NaCl solution. The stimulus evoked an increase in the plasma osmotic pressure of about 10 m-osmole/kg within 20–30 min (Brimble & Dyball, 1977). Further blood samples (0·6 ml.) were taken 30 min after the injection of hypertonic NaCl to confirm that plasma osmotic pressure had increased. Such a time course approximates more closely to that of plasma osmotic pressure changes encountered during dehydration than a rapid intravascular injection of hypertonic solution. In one test (out of thirty-three) injection of hypertonic NaCl failed to increase the plasma osmotic pressure in the second blood sample. This test was excluded from the results.

Histological techniques

At the end of each experiment, a small direct current (10 μA for 10 sec) was passed down the recording electrode to deposit iron from the electrode tip. The animal was killed with an overdose of anaesthetic and the brain dissected out and fixed in 10% formaldehyde saline containing a small quantity of potassium ferro- and ferricyanide. The resulting Prussian blue spot was then used to locate the position of the tip of the recording electrode during subsequent histological reconstruction of the hypothalamus (60 μm frozen sections). The extent of the knife cuts was determined at the same time (see Fig. 1*A*). In additional experiments, Indian ink was injected into the right common carotid artery of deafferented preparations to establish the size of the region effectively supplied with blood after total deafferentation. No obvious vascular deficit was detected.

RESULTS

Spontaneous firing rates in the supraoptic nuclei of lesioned animals

When first encountered, neurones in the supraoptic nucleus of the isolated hypothalamus fired significantly more slowly than a similar group of neurones in intact animals ($P < 0·01$; Mann–Whitney U test). Each neurone was recorded for 5 min to assess its spontaneous firing rate and the mean rates in Fig. 2 are expressed $\pm$ the standard error of the mean. All the cells recorded in intact rats were spontaneously active, but seven out of the thirteen neurones recorded from the totally deafferented hypothalamus only showed spikes when stimulated antidromically and showed no spontaneous activity (Fig. 2). However, they could be driven satisfactorily

to fire at rates above 10 Hz when stimulated in this way (see Fig. 1*B*). Neurones in the supraoptic nucleus of the partially deafferented hypothalamus (anterior or posterior cuts) fired at rates intermediate between those in totally isolated and intact hypothalami (Fig. 2). All the cells in the group of fifteen cells recorded from the posteriorly deafferented hypothalamus were spontaneously active, and two of them fired in bursts, while five out of the ten cells recorded in the supraoptic nucleus of the anteriorly deafferented hypothalamus were found to be silent. None of the latter group fired in bursts.

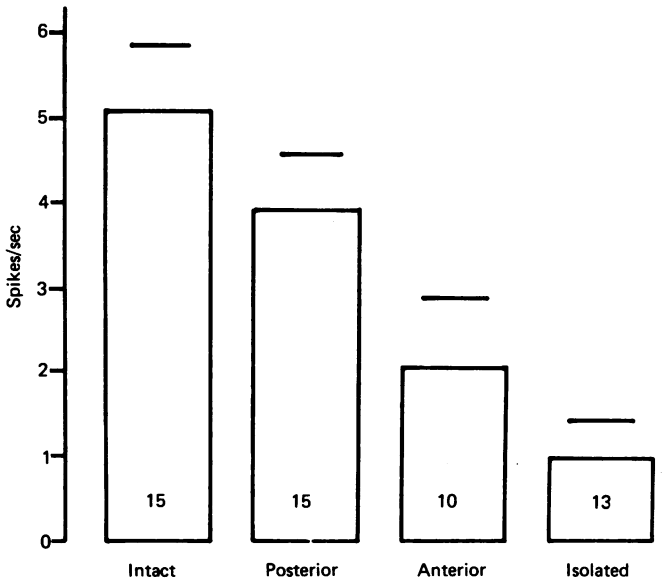

Fig. 2. The mean (±S.E. of mean) spontaneous firing rates of neurones of the supraoptic nucleus in intact animals, animals with posterior or anterior semicircular cuts and in animals with totally isolated hypothalami. The number at the bottom of each block indicates the number of values contributing to each mean.

The effects of hypertonic NaCl injections on the firing rates of supraoptic neurones

The increase in plasma osmotic pressure elicited by intraperitoneal injection of hypertonic saline was associated with a significant ($P < 0{\cdot}01$, Mann–Whitney U test) increase in the firing rate of the neurones in the supraoptic nucleus of the control rats. The increase in firing rate (which occurred at the same time as an increase in plasma osmotic pressure) reached a maximum about 30 min after the injection. Between 10 and 15 min after the injection the mean firing rate of the cells had increased by $2{\cdot}87 \pm 0{\cdot}94$ spikes/sec and 30 min after the injection it had increased by $4{\cdot}9 \pm 1{\cdot}33$ spikes/sec (see Figs. 3 and 4). By contrast, none of the five hypothalamic units outside the supraoptic nucleus which were tested and none of the seven units tested in the totally deafferented hypothalamus showed a substantial increase in firing rate when the plasma osmolality was increased by 10 m-osmole/kg. In the isolated hypothalamus the six silent cells remained silent despite the increase in plasma

osmotic pressure, while the remaining neurone showed a slightly decreased mean firing rate (0·15 spikes/sec) 30 min after the hypertonic injection.

The same lack of excitatory response to the hypertonic injection was found in the supraoptic neurones of the anteriorly deafferented hypothalamus. None of the eight cells showed an increase in firing rate which could be related to the increase in plasma

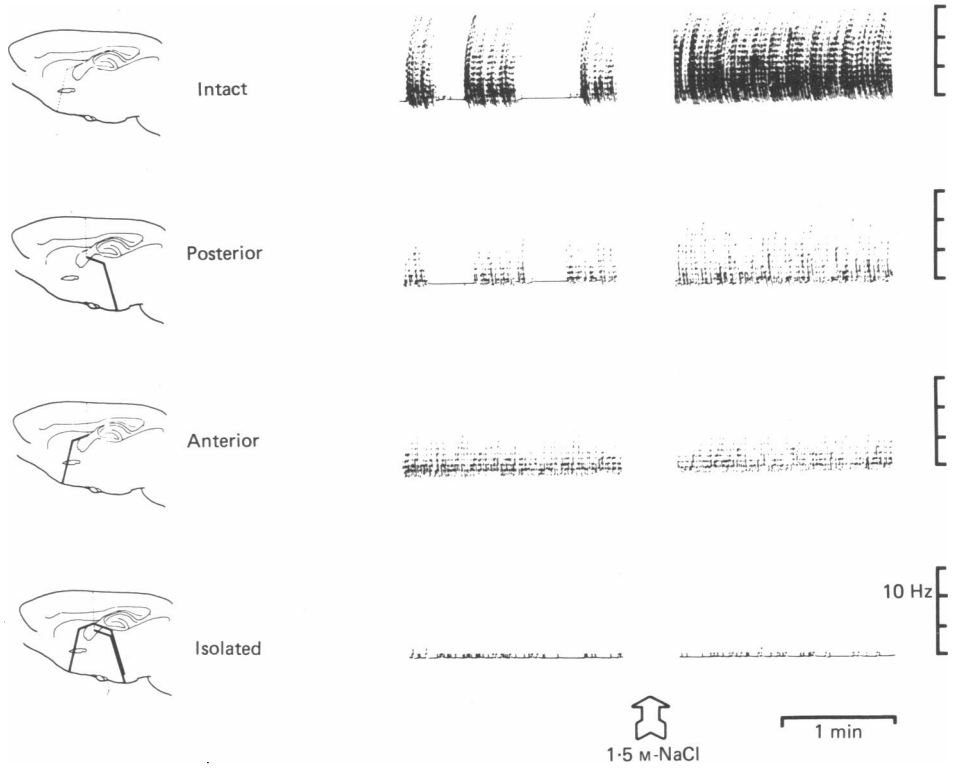

Fig. 3. Sections of rate-meter record taken before and 10–15 min after injection of hypertonic NaCl from cells in the supraoptic nucleus of intact animals, and animals with posterior or anterior semicircular cuts or a totally isolated hypothalamus. In each case hypertonic NaCl solution was injected between the section of record on the left and that on the right. Cells from intact animals and six out of nine cells with posterior cuts showed a significant excitation. Cells from the anteriorly or totally deafferented hypothalamus showed no response.

osmolality (Figs. 3 and 4). Of the four cells which were active one showed no response (Fig. 3) while three showed a decrease in firing rate.

Posterior hypothalamic deafferentation, although it reduced its magnitude, did not totally abolish the increase in firing rate elicited by the increase in plasma osmotic pressure. Twenty min after the injection the firing rate of six out of nine cells was significantly increased ($P < 0·01$, paired t test) and the mean firing rate of the group was increased by $0·95 \pm 0·34$ spikes/sec which was significantly greater ($P < 0·01$, Mann–Whitney test) than the mean firing rate shown by the neurones of the anteriorly deafferented hypothalamus at the same time.

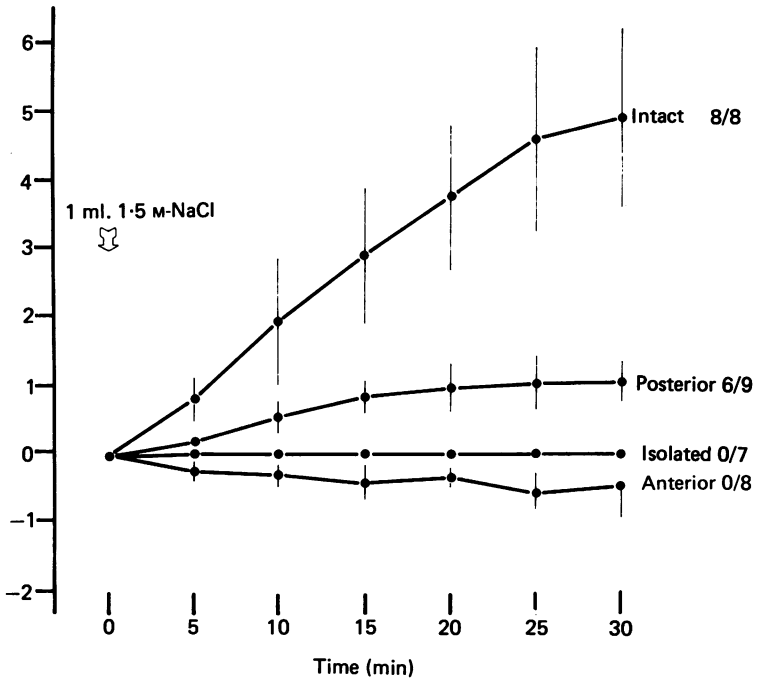

Fig. 4. The mean (±S.E. of mean) change in firing rates of cells in the supraoptic nuclei of the different types of preparation at different times after an intraperitoneal injection of 1 ml. 1·5 M-NaCl. The numbers on the right indicate the number of tests in which a significant increase in cell firing rate occurred ($P < 0.01$ paired t test).

DISCUSSION

There is now ample support for the view proposed by Verney (1947) that an increase in plasma osmotic pressure evokes a release of the antidiuretic hormone. Such a stimulus is also known to increase the firing rate of neurones in or near the supraoptic nucleus (see Hayward, 1977 for references). There is, however, less general agreement on the site and nature of the cerebral osmoreceptors (Fitzsimons, 1972; Peck & Blass, 1975; Weiss & Almli, 1975; Andersson & Olsson, 1977). The present series of experiments was thus undertaken using an extension of the concept applied by Jewell & Verney (1957); that is, to apply a stimulus for ADH release and progressively to reduce the area of the central nervous system in contact with the supraoptic nucleus so as to isolate the smallest necessary area to evoke the response. Jewell & Verney argued that this area must then contain the osmoreceptors for ADH release.

There is no doubt that in intact rats the stimulus used in these experiments both increases plasma ADH concentration (Dunn, Brennan, Nelson & Robertson, 1973) and increases the firing rate of cells in the supraoptic nucleus (Brimble & Dyball, 1977). We were able to confirm the latter results. We also showed that acute deafferentation reduced the firing rate of the cells in the supraoptic nucleus and that total or anterior isolation of the hypothalamus leads to a greater decrease in firing rate than posterior isolation. However, it is possible that the cells in the isolated hypothalamus were not

sufficiently viable to maintain normal firing rates and responsiveness. We feel that this is unlikely since the cells show no gross morphological change after deafferentation. Morris & Dyer (1971), and Dyer, Dyball & Morris (1973) have shown that the ultrastructure of secretory terminals and capillary endothelium in islands of this size appears normal, even after 48 hr. Furthermore, not all cells in the isolated hypothalamus fire slowly. In some regions (but not the paraventricular nucleus (Dyball & Dyer, 1971)), relatively fast spontaneous activity is encountered (Cross & Dyer, 1971). It is true that the technique for preparation of the islands was not the same as that used by Cross & Dyer (1971); the present method was employed to make partial isolation possible, but the blood supply of this part of the central nervous system is from below and would not have been disturbed by either method. Furthermore, the neurones in the totally isolated or anteriorly isolated hypothalamus appeared to be capable of responding to antidromic stimulation at 10 Hz (Fig. 1). We think that the reduced spontaneous activity in the partly or totally isolated hypothalamus was due to a reduced excitatory input. The results of Hatton, Armstrong & Gregory (1978) and Haller, Brimble & Wakerley (1978) which showed a reduced activity of cells in the supraoptic nucleus in hypothalamic slice preparations *in vitro* support this suggestion. The possibility remains, however, that the cuts exposed the neurones of the supraoptic nucleus to a previously masked inhibitory input from another site within the island, for example the septum (Poulain, Ellendorff & Vincent, 1980).

Our results also show that the integrity of the input to the supraoptic nucleus from the anterior was necessary to obtain an excitatory response when plasma osmolality was raised. It is possible that the technique used could have led to oedema of the tissue and that the oedemal fluid might have constituted a barrier to the diffusion of NaCl. This appears unlikely, however, since any barrier to sodium would probably have constituted a barrier to oxygen and other metabolites and led to cell death. Accordingly it appears likely that at least part of the osmosensitivity of the supraoptic nucleus is due to inputs from outside the nucleus.

It is not clear why our results are inconsistent with those of Leng (1979), but it is possible that the nature of the stimulus was not the same in the different series of experiments. Leng (1979), using an acute stimulus, found excitation of nine out of thirty cells outside the supraoptic nucleus but Brimble & Dyball (1977), using the same prolonged stimulus as employed here, failed to excite such cells. All five cells outside the nucleus tested in this series of experiments also failed to response. Sladek & Knigge (1977*a*, *b*) applied a stimulus with a similar time course to ours and showed that there was a significant release of vasopressin, but the tissue block they used may not have included the inhibitory area of the septum (see above) so that they may have observed an osmotic reponse which was inhibited in our preparation. Despite this, the observation (Sladek & Knigge, 1977*b*; Sladek & Joynt, 1978) that osmotic release of vasopression can be blocked by anticholinergic agents suggests that the neurosecretory cells are not themselves the osmoreceptors.

Mason (1980) showed in a brain slice preparation of the supraoptic nucleus that the firing rate and e.p.s.p. frequency of neurones in the supraoptic nucleus increased when the osmolality of the medium was increased. A relatively slowly applied and sustained stimulus resulted in a reproducible depolarization and an increase in the

firing rate of the cells. These results appear inconsistent with ours, although an increased e.p.s.p. frequency does imply increased excitatory input to the neurosecretory cells. Perhaps the most likely explanation of the inconsistency is that there are both local osmoreceptors in the supraoptic nucleus and more distant osmoreceptor cells in a number of extrahypothalamic sites. To obtain a consistent excitation when plasma osmolality is increased in the anaesthetized animal *in vivo* the integrated action of both supraoptic and extrahypothalamic systems may be required, whereas small differences in the resting potential of the cells *in vitro* may allow any local effects to manifest themselves. It is unlikely that responsiveness is directly related to initial firing rate because in intact animals slow firing cells frequently show large responses.

The present results, like those of Hatton *et al.* (1978) and Sladek & Joynt (1978), strongly suggest that to obtain an increase in the firing rate of the supraoptic neurones in response to an increase in the plasma osmotic pressure some input from cells other than the neurosecretory cells is required. In our experiments, the integrity of the anterior and dorsal nervous inputs to the hypothalamus was essential if the osmotic response were to be seen. No increase in firing rate was elicited by hypertonic injection when such afferents were interrupted in the anteriorly deafferented or totally deafferented hypothalamus. Although we cannot exclude the possibility that the effect of supraoptic osmoreceptor stimulation was masked in our preparation, we suggest that there is an important group of osmoreceptors which are not situated in the supraoptic nucleus but in more rostrally situated cerebral structures. Additional inputs to the supraoptic nucleus are required, however, to allow them to exert their full effect.

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REFERENCES

ANDERSSON, B. & OLSSON, K. (1977). Evidence for periventricular sodium-sensitive receptors of importance in the regulation of ADH secretion. In *Neurohypophysis*, ed. MOSES, A. M. & SHARE, L., pp. 118–127. Basel, New York: S. Karger.

BRIMBLE, M. J. & DYBALL, R. E. J. (1977). Characterization of the responses of oxytocin- and vasopressin-secreting neurones in the supraoptic nucleus to osmotic stimulation. *J. Physiol.* **271**, 253–271.

BRIMBLE, M. J., DYBALL, R. E. J. & FORSLING, M. L. (1978). Oxytocin release following osmotic activation of oxytocin neurones in the paraventricular and supraoptic nuclei. *J. Physiol.* **278**, 69–78.

CLEMENTE, C. D., SUTIN, J. & SILVERSTONE, J. T. (1957). Changes in electrical activity of the medulla on the intravenous injection of hypertonic solutions. *Am. J. Physiol.* **188**, 193–198.

CROSS, B. A. & DYER, R. G. (1971). Unit activity in rat diencephalic islands – the effect of anaesthetics. *J. Physiol.* **212**, 467–481.

DUNN, F. L., BRENNAN, T. J., NELSON, A. E. & ROBERTSON, G. L. (1973). The role of blood osmolality and volume in regulating vasopressin secretion in the rat. *J. clin. Invest.* **52**, 3213–3219.

DYBALL, R. E. J. & DYER, R. G. (1971). Plasma oxytocin concentration and paraventricular neurone activity in rats with diencephalic islands and intact brains. *J. Physiol.* **216**, 227–235.

DYBALL, R. E. J. & PRILUSKY, J. (1980). The supraoptic nucleus is not osmoresponsive in the isolated mediobasal hypothalamus. *J. Physiol.* **300**, 32*P*.

DYER, R. G., DYBALL, R. E. J. & MORRIS, J. F. (1973). The effect of hypothalamic deafferentation upon the ultrastructure and hormone content of the paraventricular nucleus. *J. Endocr.* **57**, 509–516.

FITZSIMONS, J. T. (1972). Thirst. *Physiol. Rev.* **52**, 468–561.

HALASZ, B. & PUPP, L. (1965). Hormone secretion of the anterior pituitary gland after physical interruption of all nervous pathways to the hypophysiotrophic area. *Endocrinology* **77**, 553–562.

HALLER, E. W., BRIMBLE, M. J. & WAKERLEY, J. B. (1978). Phasic discharge in supraoptic neurones recorded from hypothalamic slices. *Expl Brain Res.* **33**, 131–134.

HATTON, G. I., ARMSTRONG, W. E. & GREGORY, W. A. (1978). Spontaneous and osmotically-stimulated activity in slices of rat hypothalamus. *Brain Res. Bull.* **3**, 497–508.

HAYWARD, J. N. (1977). Functional and morphological aspects of hypothalamic neurons. *Physiol. Rev.* **57**, 574–658.

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.* B **240**, 197–324.

LENG, G. (1979). Responses of supraoptic neurones to local hypertonic saline in the rat. *J. Physiol.* **296**, 62–63*P*.

MALMO, R. B. (1976). Osmosensitive neurons in the rat's dorsal midbrain. *Brain Res.* **105**, 105–120.

MASON, W. T. (1980). Intracellular recordings from osmosensitive neurones in the rat supraoptic nucleus *in vitro*. *J. Physiol.* **303**, 56*P*.

MORRIS, J. F. & DYER, R. G. (1971). Neurosecretion in the paraventricular nucleus of diencephalic islands. *J. Anat.* **110**, 139–140.

PECK, J. W. & BLASS, E. M. (1975). Localisation of thirst and antidiuretic osmoreceptors by intracranial injections in rats. *Am. J. Physiol.* **228**, 1501–1509.

POULAIN, D. A., ELLENDORFF, F. & VINCENT, J. D. (1980). Septal connections with identified oxytocin and vasopressin neurones in the supraoptic nucleus of the rat. An electrophysiological investigation. *Neuroscience* (In the Press).

SAWYER, C. H. & GERNANDT, B. E. (1956). Effects of intracarotid and intraventricular injections of hypertonic solutions on electrical activity of the rabbit brain. *Am. J. Physiol.* **185**, 209–216.

SLADEK, C. D. & JOYNT, R. J. (1978). Cholinergic involvement in the osmotic control of vasopressin release by the organ cultured rat hypothalamoneurohypophysial system. *Neurology* **28**, 366.

SLADEK, C. D. & KNIGGE, K. M. (1977*a*). Osmotic control of vasopressin release by rat hypothalamo-neurohypophysial explants in organ culture. *Endocrinology* **101**, 1834–1838.

SLADEK, C. D. & KNIGGE, K. M. (1977*b*). Cholinergic stimulation of vasopressin release from the rat hypothalamo–neurohypophyseal system. *Endocrinology* **101**, 411–420.

SUNDSTEN, J. W. & SAWYER, C. H. (1961). Osmotic activation of neurohypophysial hormone release in rabbits with hypothalamic islands. *Expl Neurol.* **4**, 548–561.

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

WEISS, C. S. & ALMLI, C. R. (1975). Lateral preoptic and lateral hypothalamic units: in search of the osmoreceptor for thirst. *Physiol. & Behav.* **15**, 713–722.

WOODS, J. W., BARD, P. & BLEIER, R. (1966). Functional capacity of the deafferented hypothalamus: water balance and responses to osmotic stimuli in the decerebrate cat and rat. *J. Neurophysiol.* **29**, 751–767.