J. Physiol. (1969), **201**, 711–722
With 7 text-figures
Printed in Great Britain

ELECTRICAL ACTIVITY IN THE SUPRAOPTIC AND PARAVENTRICULAR NUCLEI ASSOCIATED WITH NEUROHYPOPHYSIAL HORMONE RELEASE

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(*Received* 10 *October* 1968)

SUMMARY

1. Unit recordings were made from the hypothalamus in anaesthetized male rats using steel or glass micro-electrodes.

2. Stimuli which are known to release vasopressin and oxytocin (electrical stimulation of the central end of the severed right vagus nerve and intracarotid injection of $CaCl_2$ solution) also excite units in the supraoptic and paraventricular nuclei. In addition, these units are excited by intracarotid injections of carbachol, acetylcholine and NaCl (5%) which are less effective stimuli for vasopressin release.

3. These stimuli also excite units from which potentials can be evoked by stimulation of the pituitary stalk and which are likely to be neurosecretory neurones. Neurosecretory neurones can conduct electrical impulses like other less specialized nerve cells. Estimations of conduction velocity range between 0·4 and 1·3 m/sec.

4. The proportion of units in the supraoptic and paraventricular nuclei which are excited and the degree of excitation corresponds approximately to the amount of vasopressin released by the stimuli. In addition, excitation of the supraoptic nucleus seems to be more directly associated with vasopressin release and excitation of the paraventricular nucleus with oxytocin release.

INTRODUCTION

A considerable body of evidence has been available for a number of years to support the hypothesis that an increase in the activity of neurones in the supraoptic and paraventricular nuclei of the hypothalamus leads to the release of neurohypophysial hormones (Scharrer & Scharrer, 1954;

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Harris, 1955; Cross & Silver, 1966). However, a great deal of this evidence is somewhat indirect.

In 1959 Cross & Green showed that action potentials could be recorded from supraoptic neurones in rabbits. More recently it has been shown that the hypothalamic neurosecretory cells of goldfish (identified by antidromic stimulation of the pituitary stalk) are capable of conducting impulses like other less specialized neurones, and that these cells are sensitive to osmotic stimulation (Kandel, 1964). Yagi, Azuma & Matsuda (1966) made similar observations in rats and were able to increase the spontaneous firing rate of neurones in the supraoptic nucleus by intracarotid infusions of hypertonic sodium chloride solution.

Brooks, Ishikawa, Koizumi & Lu (1966) measured the discharge rate of neurones in the paraventricular nucleus of lactating cats. They showed that stimuli which caused a milk-ejection response also increased the firing rate of these neurones and so were able to demonstrate a direct functional relationship between paraventricular neurones and oxytocin release. However, although stimuli which are likely to release vasopressin have been shown to excite neurones in the supraoptic and paraventricular nuclei (see Cross & Silver, 1966) no parallel experiments have been carried out to show that under similar experimental conditions the concentration of vasopressin in circulating blood increases.

Dyball (1968) investigated the effects of a number of stimuli on the concentrations of vasopressin in blood from the right external jugular vein of rats. The purpose of the present investigation was to discover whether or not these stimuli increased the firing rate of neurones in the supraoptic and paraventricular nuclei so that a direct functional relationship could be established between these nuclei and vasopressin release.

Dyball (1968) also measured the effects of two of the stimuli on the jugular blood concentration of oxytocin. Lederis (1962) found that the proportions of vasopressin and oxytocin which can be extracted from homogenates of the tissues suggest that the supraoptic nucleus is more concerned with vasopressin release and the paraventricular with oxytocin. There is also a considerable amount of evidence which suggests that vasopressin and oxytocin can be released independently (for example, Beleslin, Bisset, Haldar & Polak, 1967; Bisset, Hilton & Poisner, 1967; Dyball, 1968). It was, therefore, hoped that the investigation might also give some information on whether or not the two nuclei are functionally specialized.

METHODS

The experiments were performed on 115 male albino rats of between 200 and 250 g in weight. They were anaesthetized with intraperitoneal urethane (25%, w/v, solution, 0·6 ml. per 100 g rat) and given atropine sulphate (1 mg per 10 g rat) subcutaneously. As

soon as anaesthesia had developed, two fine polyethylene cannulae were inserted into the right common carotid artery through a ventral incision in the neck. One of these was directed cranially so that injection could be made into the cerebral circulation and the other towards the heart. This second cannula was connected to a pressure transducer and a Gilson recorder so that the blood pressure could be monitored and the condition of the animal assessed throughout the experiment. The trachea was cannulated and a small bipolar stimulating electrode attached to the cranial end of the severed right vagus nerve through the same incision which was then closed. The animal was fixed rigidly in the head holder of a Baltimore Instrument Company stereotaxic instrument. A skin incision was made over the region of the cerebral cortex and the dorsal surface of the brain in the region of the bregma exposed through a small trephine hole.

Insl-X coated, steel micro-electrodes (Zeballos, Wang, Koizumi & Brooks, 1967) were introduced into the supraoptic and paraventricular nuclei, according to the stereotaxic co-ordinates of de Groot (1959), for the recording of unit activity. The signal picked up from the micro-electrode was amplified by a condensor-coupled preamplifier with a 2 msec time constant (Tektronix 122) and displayed on an oscilloscope (Tektronix 502A). Recordings were made by photographing the trace of the oscilloscope with a Grass kymograph camera. In addition, the output from the preamplifier was fed into a pulse counting device (developed by Dr J. Ushiyama in this laboratory) and recorded on a Honeywell Visicorder. Using this instrument a convenient recording of the number of spikes in unit time could be made.

The position of the recording electrode was established at the end of each experiment by the Prussian Blue spot method (Brooks *et al.* 1966). To avoid confusion, only one recording track and blue spot was made on each side of the brain. Figure 1 shows typical sections with large lesions in the supraoptic nucleus (Fig. 1*A*) and paraventricular nucleus (Fig. 1*B*). Since units in the vicinity of, but peripheral to, the histologically identifiable paraventricular nucleus have been shown to be associated with milk-ejection (Brooks *et al.* 1966), all units within 1 mm of the centre of each nucleus were, for the purposes of analysis, treated as if they were supraoptic or paraventricular neurones.

In a small number of experiments, glass capillary micro-electrodes (resistance 15–20 MΩ) and a cathode follower (Bak standard wide band electrometer) were used for recording unit discharges. To identify neurosecretory cells in these experiments, a small bipolar steel stimulating electrode was inserted into the neural lobe from the dorsal side and the recording electrode advanced until a unit was found from which a spike potential could be evoked by stimulation of the neural lobe. Pulses of 0·07–2·0 msec and between 10 and 25 V were applied in such antidromic stimulations. The exact position of the stimulating electrode was determined by the Prussian Blue spot method at the end of each experiment. Successful recordings were made in this way from nine units, all of which were in the supraoptic nucleus.

After a unit had been identified in this way or by its anatomical position, its discharge rate was monitored for a control period of 2 or 3 min. The stimuli which had been used in the earlier work (Dyball, 1968) were then applied (with minor modification in order to make unit recording easier) in random order at approximately 5 min intervals. (The effects of the stimuli persisted for between 1 and 4 min.) The discharge rate was monitored constantly until all the stimuli had been completed. The stimuli used were intracarotid injection of acetylcholine chloride (0·1 mg dissolved in 0·1 ml. isotonic NaCl solution followed by 0·1 ml. of isotonic NaCl solution), carbachol chloride (in the same dose as acetylcholine), $CaCl_2$ solution (0·15 ml. of an M/10 solution followed by 0·05 ml. isotonic NaCl solution), hypertonic NaCl solution (0·15 ml. of a 5% solution of NaCl followed by 0·05 ml. of isotonic NaCl), isotonic NaCl solution (0·2 ml.) and electrical stimulation of the central end of the severed right vagus nerve (for 15 sec with square waves of 2 msec duration at 15 V and 100 c/s). All the solutions were injected at a constant rate (0·1 ml. per 10 sec) with tuberculin syringes.

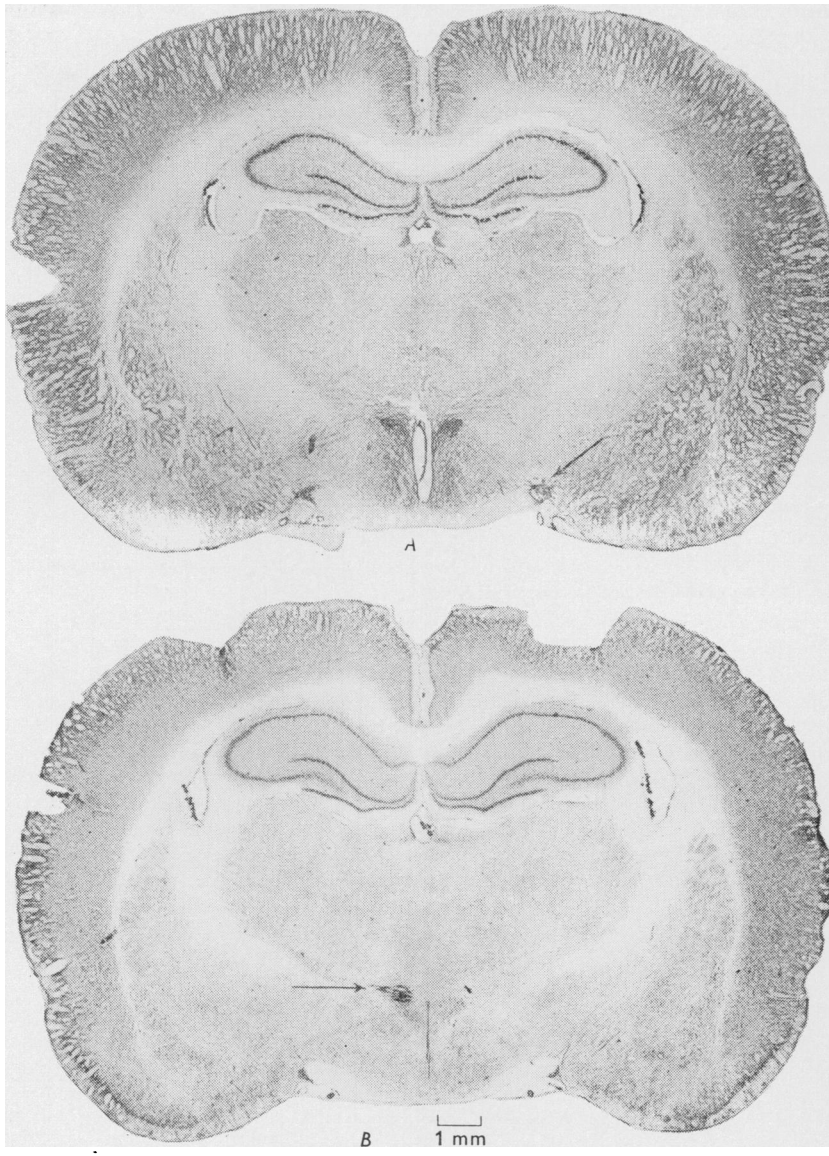

Fig. 1. Typical sections of the rat brain showing large lesions (arrowed) *A*, in the supraoptic nucleus and *B*, in the paraventricular nucleus.

RESULTS

The tracings of Fig. 2 are records obtained from the oscilloscope showing effects on the rate of discharge of typical supraoptic units of an intracarotid $CaCl_2$ injection, vagal stimulation and intracarotid injections of

isotonic NaCl solution, carbachol and acetylcholine. The upper tracing in each case represents a control period and the lower tracing a period after stimulation or injection. Figure 3 shows the changes in discharge rate of a typical supraoptic neurone after intracarotid injection of $CaCl_2$ solution, NaCl (5 %) solution and carbachol. The responses were recorded by the pulse counting device mentioned earlier. Each step on the record represents 1 spike potential and each 'staircase' or vertical line represents the

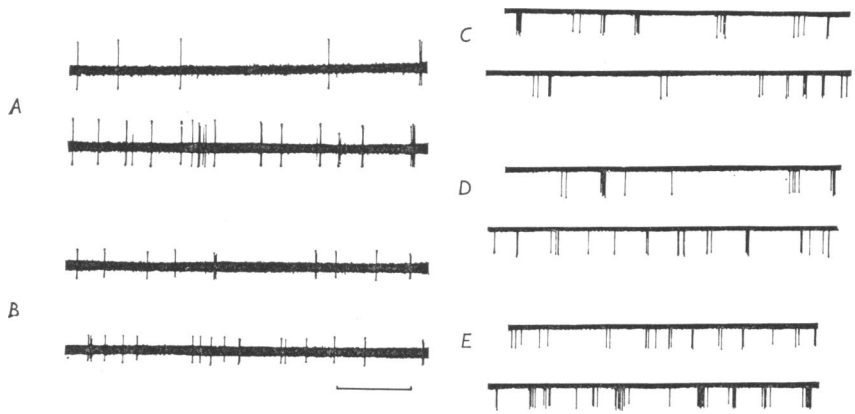

Fig. 2. Photographs of typical oscilloscope tracings from supraoptic units. The upper trace represents a control period in each case and the lower trace a period after stimulation of the vagus or injection of drugs. Responses shown are to *A*, injection of M/10 $CaCl_2$ solution; *B*, vagal stimulation; *C*, isotonic NaCl; *D*, carbachol; and *E*, acetylcholine injections. Time mark, 5 sec.

number of spikes in a 5 sec period. It can be seen from these records that units in the supraoptic nucleus can be accelerated by vagal stimulation, and intracarotid injections of $CaCl_2$ solution, carbachol, acetylcholine and NaCl (5 %) solution but not by an isotonic NaCl solution. Frequently, small fluctuations in blood pressure occurred during injection or stimulation but these changes did not appear to be directly related to alterations in the rate of discharge of the units.

Similar recordings were made from a total of ninety-seven supraoptic units, fifty-six paraventricular units and forty-eight neurones in the dorsal hypothalamus or thalamus lying some distance away from the supraoptic and paraventricular nuclei, although it was not possible to record from every unit for a long enough time to investigate the effects of all the stimuli. The information from all the units in each group was then assembled in an attempt to assess the overall effects of the different stimuli on the supraoptic and paraventricular nuclei. For the purposes of analysis a neurone was considered to have been excited if its firing rate observed between 20 and 30 sec after the end of stimulation or injection

was 20% or more above the resting rate. It was judged to have been depressed if its firing rate during this period was 20% or more below the resting rate. The resting rate was calculated from the mean of the firing rates in two 20 sec periods, one immediately before the stimulation or injection and the other approximately 5 min after it. The period between 20 and 30 sec after stimulation or injection was chosen because in the earlier work on vasopressin release (Dyball, 1968) blood samples were taken 30 sec after the end of the stimulation or injection. A change of 20% in the discharge rate was chosen arbitrarily but similar patterns of responses emerged if changes of 10 or 30% were used.

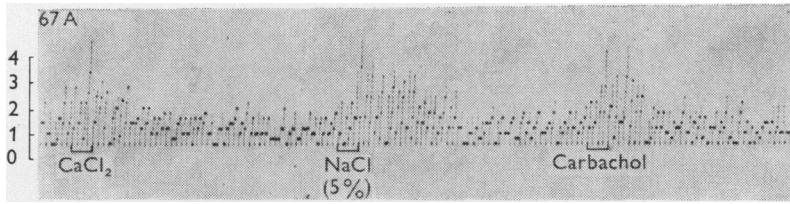

Fig. 3. Typical record from the pulse counting device. Each step represents one spike and each staircase represents the number of spikes in a 5 sec period. The scale on the left indicates the number of spikes/sec. The responses of a typical supraoptic unit to $CaCl_2$ solution, NaCl (5%) solution and carbachol are shown.

Although there is probably some degree of specialization of function between the supraoptic and paraventricular nuclei it is likely that the paraventricular nucleus is, to some extent, concerned with vasopressin release. Accordingly, for the purposes of initial analysis, the responses of the units in the supraoptic and paraventricular nuclei were added together. These results are shown in Fig. 4.

It can be seen that the stimuli (vagal stimulation, $CaCl_2$ injection, carbachol injection and acetylcholine injection) which released vasopressin in the earlier study (Dyball, 1968) also excited neurones in the paraventricular and supraoptic nuclei. Some neurones were also excited by intracarotid 5% NaCl injection which appeared to be an ineffective stimulus for vasopressin release. Intracarotid injections of isotonic NaCl were much less effective in exciting the neurones and in releasing vasopressin.

Figure 5 shows, in separate columns, the proportions of the units in the supraoptic nucleus, the paraventricular nucleus and the dorsal hypothalamus which responded to the different stimuli; the dorsal hypothalamic units may be considered as a control group. Approximately the same proportion of units in the supraoptic and paraventricular nuclei was excited by the different stimuli but $CaCl_2$ injection excited 11% and carbachol 27% more units in the paraventricular nucleus than in the supraoptic nucleus. However, units in the dorsal hypothalamus or thala-

mus seemed to be more difficult to excite. Each injection depressed approximately 20% of the units in the supraoptic nucleus and dorsal hypothalamus but fewer of those in the paraventricular nucleus.

It is thought that the rate of discharge of supraoptic and paraventricular neurones is related to the release of neurohypophysial hormones. Accordingly, the effects of the different stimuli on the discharge rates of all the units in the supraoptic and paraventricular nuclei were calculated. Figure 6

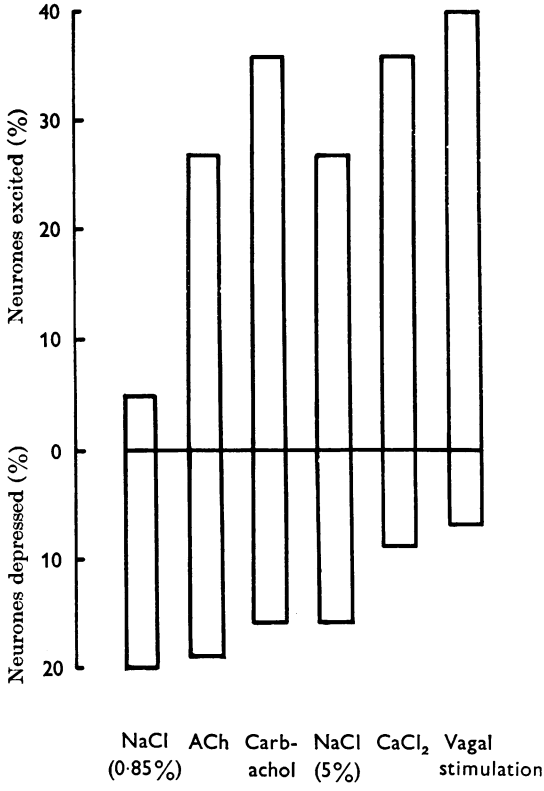

Fig. 4. The proportion of units excited and depressed by the different stimuli (responses of units from supraoptic and paraventricular nucleus combined).

shows the mean increase above or decrease below the control rate of discharge of all these units, during and for 1 min after stimulation or injection, *A* in the supraoptic nucleus, *B* in the paraventricular nucleus and *C* in the two nuclei combined. As had been expected from the results of earlier investigations (Cross & Silver, 1966), a great variation was found in the control discharge rate of the units (from 1 spike/10 sec to 72 spikes/sec with a mean of 7·5) so that no estimate of the standard errors of these means is shown. However, an increase above the control rate occurred

after vagal stimulation and all the injections with the exception of isotonic NaCl. Examination of the distribution of these means around the means for the control discharge rates in Fig. 6*C* with the supraoptic and paraventricular units combined suggests that the distribution for all the stimuli (again with the exception of isotonic NaCl injection) is highly

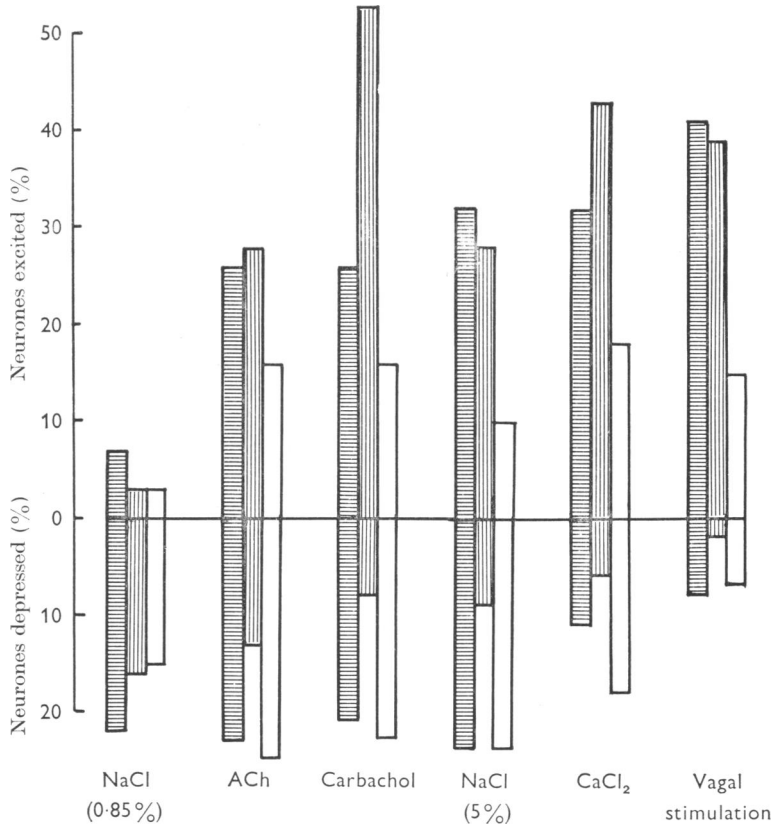

Fig. 5. The proportions of the units in the supraoptic nucleus, the paraventricular nucleus and the dorsal hypothalamus which were excited and depressed by the stimuli. ▤ supraoptic units, ▥ paraventricular units, □ dorsal hypothalamic units.

unlikely to have occurred by chance ($P = < 0{\cdot}05\,\%$ by the χ^2 test). In addition, the more powerful stimuli for vasopressin release tend to cause greater increases in discharge rate.

In Fig. 7 are shown spike potentials evoked in two supraoptic units by stimulation of the pituitary stalk or neural lobe (Fig. 7*B*, *D*) together with recordings of the firing rates of the nerve cells during and after intracarotid carbachol injection (Fig. 7*A*, *C*). It can be seen that these units are excited by carbachol injection in the same way as the units

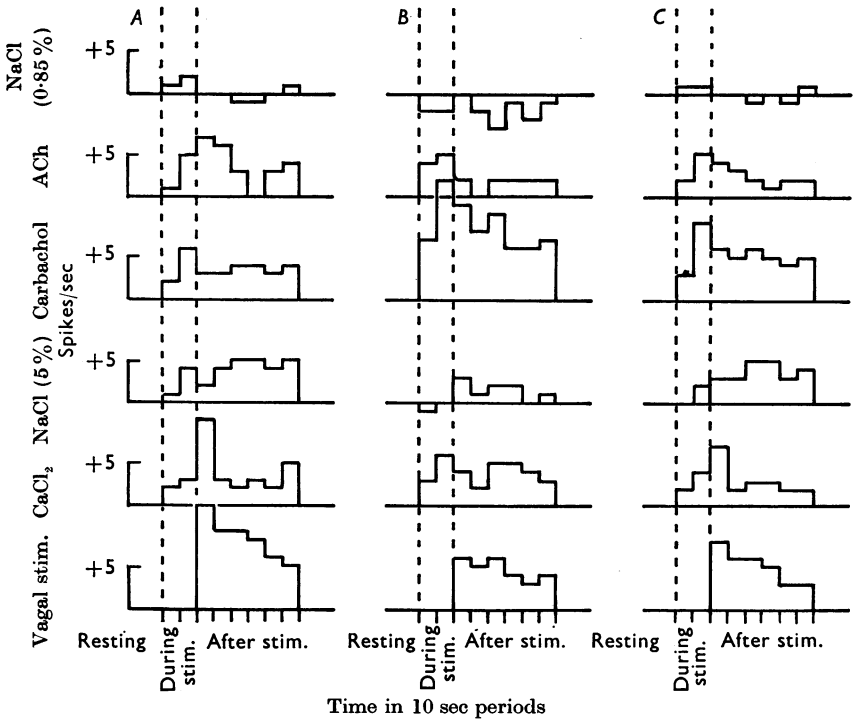

Fig. 6. The changes in mean firing rates of units during and after stimulation. *A*, supraoptic nucleus; *B*, paraventricular nucleus; *C*, paraventricular and supraoptic nuclei combined.

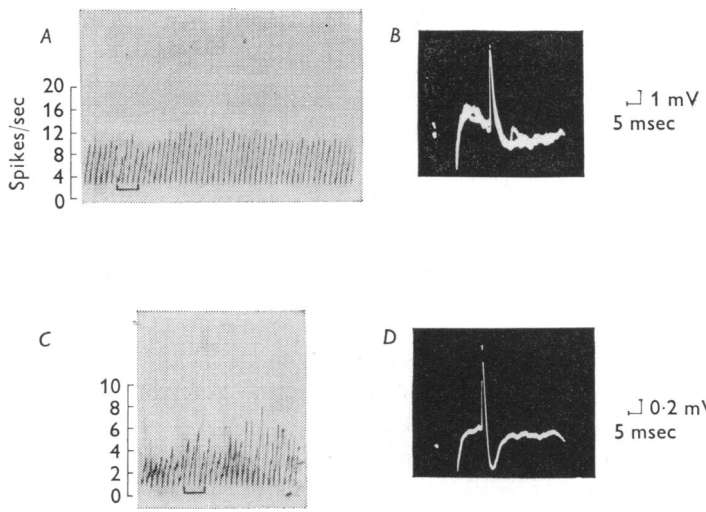

Fig. 7. Recordings from the pulse counter of two supraoptic units during carbachol injection (*A* and *C*) together with photographs of the spike potentials evoked in them by stimulation of the pituitary stalk (*B* and *D*).

identified by histological criteria. The trace in Fig. 7*B* shows five consecutively evoked spikes superimposed; each has exactly the same latency indicating that the spikes photographed are highly unlikely to have been spontaneous.

It is not easy to calculate the conduction velocity of the neurosecretory nerve fibres since the positions of the recording electrodes were not accurately known (although the position of the stimulating electrode was determined by the Prussian Blue spot method mentioned earlier). However, if the distance between them is calculated from the stereotaxic co-ordinates, the conduction velocities for the different cells range between 0·4 and 1·3 m/sec.

DISCUSSION

The results reported demonstrate that a number of neurones in the regions of the supraoptic and paraventricular nuclei can be excited by stimuli which have been shown to release vasopressin. This strongly suggests a causal relationship between the two events. Since the majority of these units were classified as supraoptic or paraventricular units simply by their position, it is likely that not all of them were neurosecretory neurones. Some of them may have been neurones related to but not part of the neurosecretory system. However, units which were identified as neurones of the supraopticohypophysial tract by antidromic stimulation of the pituitary stalk also responded in a similar way.

The experiments in which the pituitary stalk was stimulated also confirm the findings of Yagi *et al.* (1966) that the neurosecretory cells of mammals can be excited and conduct impulses in a similar way to other neurones. The estimated value for the conduction rate of neurosecretory neurones (between 0·4 and 1·3/sec) is also consistent with the results of other workers (Ishikawa, Koizumi & Brooks, 1966; Yagi *et al.* 1966).

The present series of experiments is concerned with the effect of a number of stimuli on unit activity in the neurones of the supraoptic and paraventricular nuclei. In a previous series of experiments (Dyball, 1968), the amount of vasopressin released into the blood by the same stimuli in the same species was determined. It can be seen that there is a general correlation between the amount of vasopressin released by each stimulus and both the number of units excited and the degree to which they are excited. A more exact correlation could not be expected since the two series of experiments were carried out in different laboratories. The most powerful stimuli for vasopressin release, vagal stimulation and $CaCl_2$ injection, excited 40 and 36 %, respectively, of the units tested (see Fig. 4). The next most effective stimulus, carbachol injection, also excited 36 % of the units although it was very much less effective in releasing vasopressin. However, it should be

noted that carbachol injection inhibited about twice as many of the units as vagal stimulation and $CaCl_2$ injection and this may imply that it is a less effective excitatory stimulus. In addition, whereas the same dose of carbachol was given in both series of experiments, in the interests of successful unit recording, only about one-sixth of the originally used volume of $CaCl_2$ was injected and vagal stimulation was carried out for 15 and not 30 sec. It might be expected, therefore, that carbachol would appear to be a more effective stimulus in this series of experiments.

NaCl (5 %) injection excited 27 % of the units although it had not been found to cause a detectable increase in the jugular blood concentration of vasopressin. It is not clear why this is so but it may have been due to the different volumes of solution injected in the two series of experiments (about one-fifth of the volume used in the earlier work was injected in these experiments). Isotonic NaCl injection depressed 20 % of the units tested and it is likely that the reasons for this depression are non-specific; possibly the volume of the solution injected was of significance. If this is the case, the larger volume of solution injected in the earlier work may have masked the comparatively mild hypertonic stimulus.

There is good evidence to suggest that the supraoptic nucleus is concerned mainly with vasopressin release and the paraventricular with oxytocin (see Lederis, 1962). The results of these experiments although not clear cut, are consistent with this hypothesis. Dyball (1968) reported that the plasma concentration of oxytocin was greater than that of vasopressin after $CaCl_2$ injection in three out of four experiments. In the experiments described in this paper, 11 % more paraventricular units than supraoptic units were excited by $CaCl_2$ injection (see Fig. 5), although the difference between the effects on the mean discharge rates is not obvious (see Fig. 6*A*, *B*). On the other hand, vagal stimulation seemed to be more effective in exciting supraoptic than paraventricular units (see Fig. 6*A*, *B*). This reflects what might have been expected from the experiments on hormone release since after vagal stimulation the plasma concentration of vasopressin was higher than that of oxytocin in three out of three experiments.

Intracarotid NaCl (5 %) excited supraoptic units to a greater extent than those in the paraventricular nucleus (see Fig. 6*A*, *B*). If the supraoptic nucleus is more concerned with vasopressin release than the paraventricular, this finding might have been expected since vasopressin is more likely to be concerned with the regulation of blood osmolarity than oxytocin. These results confirm the findings of Brooks *et al.* (1966) in cats.

As judged by mean firing rates, carbachol injection exerted a more powerful excitatory effect on the paraventricular nucleus than on the upraoptic, and acetylcholine a more powerful effect on the supraoptic

nucleus than on the paraventricular (cf. Fig. 6). However, since no oxytocin estimations were made after these stimuli, the differences are difficult to interpret.

The authors are much indebted to Dr C. McC. Brooks for much useful advice and criticism during the course of the experiments and to Mrs Maria-Eliana Zeballos for her help in the preparation of histological material.

The work was supported by a grant from the USPHS NB6537-02. One of the authors (R.E.J.D.) was also supported by a Wellcome Research Travel Grant.

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