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OXYTOCIN AND ADH SECRETION IN RELATION TO ELECTRICAL ACTIVITY IN ANTIDROMICALLY IDENTIFIED SUPRAOPTIC AND PARAVENTRICULAR UNITS

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SUMMARY

1. Electrical recordings were made from antidromically identified supraoptic and paraventricular units during intracarotid injections of hypertonic and isotonic sodium chloride solutions in rats.

2. The blood concentrations of vasopressin and oxytocin were estimated by bio-assay before and at different intervals after similar injections.

3. Although a significant change in the action potential activity of the supraoptic nucleus was associated with hormone release, the results were not entirely consistent with a simple relationship between action potential activity and hormone secretion. Firstly, although some units were excited by the stimulus a substantial number were inhibited. Secondly, the blood concentration of the hormones, particularly ADH, remained elevated for longer than might have been expected if additional hormone had ceased to be secreted as soon as firing rates had returned to control values.

4. There were substantial differences between the initial blood concentrations of vasopressin and oxytocin but the firing rates of units in the supraoptic and paraventricular nuclei appeared to be the same.

5. Although significantly less paraventricular than supraoptic units were affected by hypertonic injections the blood concentration of oxytocin was increased by a factor of 8 whereas that of vasopressin was increased by a factor of 2·7.

INTRODUCTION

The classical experiments of Verney (1947) clearly demonstrated the connexion between the posterior pituitary and antidiuretic hormone (ADH) release. Somewhat later Harris (1955) showed that electrical stimulation of the hypothalamo–neurohypophysial system would inhibit water diuresis and cause milk ejection. It was then established that normal

action potentials could be recorded from neurones of the supraoptic and paraventricular nuclei and that the rate of discharge of these neurones could be influenced by intracarotid injections of hypertonic sodium chloride solution, a stimulus which Verney had shown to release ADH (Cross & Green, 1959). In 1966 Brooks, Ishikawa, Koizumi & Lu found that the suckling stimulus could be associated simultaneously with milk ejection and an increased discharge rate of paraventricular units. There can be little doubt therefore that the release of ADH and oxytocin is associated with a change in the electrical activity of supraoptic and paraventricular neurones.

Recently it has become possible to identify cells of the hypothalamo–hypophysial system of rats by antidromic stimulation of the neural lobe (Yagi, Azuma & Matsuda, 1966; Dyball & Koizumi, 1969; Dyball, 1969). In addition the methods for extraction and assay of neurohypophysial hormones have been improved (Bisset, Clark, Haldar, Harris, Lewis & Rocha e Silva, 1967; Bisset, Hilton & Poisner, 1967). It seemed important therefore to apply these techniques to a more critical investigation of the nature of the relationship between the electrical and secretory properties of neurosecretory neurones.

METHODS

Male Wistar rats of the Porton strain, approximately 200 g in body weight, given dry pellets (Oxoid Ltd) and water *ad lib* were used throughout the investigation. They were anaesthetized with urethane (1·5 g/kg) and cannulae were inserted into the trachea, right femoral vein and right common carotid artery (towards the head). One group was then prepared for electrophysiological recording; a further cannula was inserted into the right common carotid artery towards the heart for blood pressure recording and the animal was placed in a Hoffman–Reiter hypophysectomy instrument modified for antidromic stimulation of the neurohypophysis (Dyball, 1969). Finally steel wire e.e.g. electrodes were cemented on to the parietal bones of the skull and a small trephine hole made for the insertion of the recording microelectrodes. Recordings were then made from units within the paraventricular and supraoptic nuclei, located according to the stereotaxic co-ordinates of de Groot (1959) and which could be antidromically activated (see Results) during intracarotid injections of 0·25 ml. of isotonic and 1 M solutions of sodium chloride. The recording electrodes used were electrolytically sharpened steel insect pins insulated with Insl-X (Insl-X Products Corp., Yonkers, N.Y.). Signals from these electrodes were amplified and recorded by apparatus similar to that used by Dyball & Koizumi (1969). At the end of each experiment a small DC current was passed down the recording electrode so that the position of the electrode tip during recording could be subsequently confirmed by the Prussian-blue spot method.

The second group of rats was prepared for blood collection in batches of six by the insertion of a femoral vein cannula (for the injection of 1500 i.u. heparin) and a larger cannula into the right external jugular vein for the collection of blood from the head. In each experiment 1 ml. blood samples were taken from each of the six rats. An injection of 0·7 ml. dextran solution (Dextraven, Benger Laboratories Ltd) was given via the femoral cannula which, it was hoped, would together with the 0·3 ml.

of the heparin injection, compensate for the loss of the 1 ml. blood samples. The use of dextran solution to maintain blood volume is justified in spite of its possible side effects, since similar injections were given in both control and experimental rats, and the blood concentrations of antidiuretic and milk-ejection activity following isotonic injections were not substantially raised. Intracarotid injections of 0·25 ml. of isotonic or 1 M sodium chloride solutions were given (through the cranially directed carotid cannula at a rate of 0·6 ml./min) and a second 1 ml. blood sample taken immediately or at 1, 3, 5 or 7 min after the end of the injection. The blood samples before and after injection were pooled, extracted by the method of Bisset *et al.* (1967) and assayed for milk ejection and antidiuretic activity. Antidiuretic activity was estimated in the water-loaded ethanol-anaesthetized rat using the apparatus described by Dyball, Lane & Morris (1966) and milk-ejection activity using the method of Bisset *et al.* (1967). The changes in intramammary pressure were detected by a Bell and Howell pressure transducer (type 4-327-L223) and recorded on a Bryans chart recorder (type 27000). In each case a 2 + 2 assay design was used with at least two repetitions so that 95 % confidence limits could be calculated by the method of Gaddum (1953). This form of assay has the additional advantage that for each comparison it could be established that there was no significant deviation from parallelism of the log. dose response lines for the standard preparations (synthetic arginine vasopressin kindly supplied by Hoechst AG and syntocinon, Sandoz) and the unknown solutions. This, together with the destruction of all detectable activity by incubation with sodium thioglycollate (Vogt, 1953), makes it unlikely that the activity detected was not due to oxytocin and AVP.

RESULTS

Identification of units

Recordings were made from a total of seventy-seven supraoptic and sixty-nine paraventricular units in the hypothalamus from which single action potentials could be evoked by stimulation of the neural lobe. Since the tip of the stimulating electrode lay on the floor of the skull beneath the adenohypophysis rather powerful pulses (typically 0·5 msec and 30 V : 2 mA) had to be used to ensure sufficient current spread to stimulate the pituitary stalk. Monophasic square-wave pulses were found to be more effective than biphasic pulses. A unit was not considered to be antidromically activated unless the latency of the evoked spike was constant (see Fig. 1*A*). In addition, except in the first few experiments, if the unit was spontaneously active it was not included unless the evoked spike collided with a spontaneously active one (see Fig. 1*B*, *C*).

Spontaneous discharge rates

A great variation was found in the spontaneous discharge rates of units in both paraventricular and supraoptic nuclei but there was a high proportion of slow firing units (less than 1/sec) in each nucleus. Fig. 2 shows that the firing rates of the units in the two nuclei were not normally distributed so that a simple comparison of mean firing rates could not be made. However, it can be seen that there was no major difference between the cell firing rates of the two nuclei. There were more completely inactive

units in the paraventricular nucleus than in the supraoptic nucleus (15 out of 69, compared with 9 out of 77) and more cells in the supraoptic than paraventricular nucleus fired at more than 6/sec (13 out of 77, compared with 5 out of 69) but these differences in distribution are not significant using the Kolmogorov–Smirnov test.

Fig. 1. *A*, photograph of three oscilloscope sweeps superimposed to show the constant latency of the antidromic action potential, the initial deflexion is a large stimulus artifact and the second is the antidromic potential. *B*, a train of two pulses each followed by an antidromic action potential. *C*, the same train of two impulses triggered by a spontaneous action potential. In this case the first antidromic action potential collides with the spontaneous spike and disappears but the second appears as expected (scale mark: *A*, 0·2 mV and 5 msec; *B* and *C*, 0·2 mV and 10 msec).

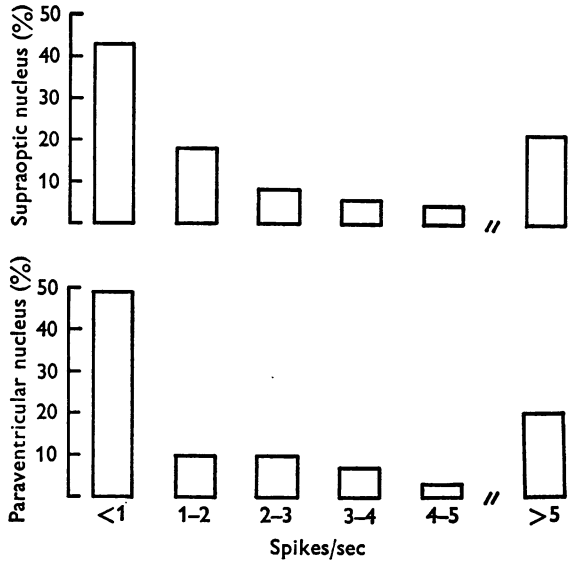

Fig. 2. Histogram showing the distribution of spontaneous discharge rates of supraoptic and paraventricular nucleus neurones.

Changes after intracarotid injections

Typically those units which were firing slowly (< 2 spikes/sec) were excited and those units which were firing fast (> 2 spikes/sec) were inhibited by intracarotid hypertonic injections (see Fig. 3*A*, *B*). Also shown are the e.e.g. and blood pressure changes during the injections. Usually no substantial change in either blood pressure or e.e.g. occurred after isotonic injections but although there were always changes in blood pressure after hypertonic injections, there was no obvious relationship between these and the firing rate. Occasionally desynchronization of e.e.g. was observed after hypertonic injections.

In some of the experiments, when it was possible to record from a single unit for a long period of time, the spontaneous discharge rate of the unit either slowed down or speeded up between responses. In this situation the same unit tended to be accelerated by hypertonic sodium chloride when firing slowly and to be inhibited when firing fast. This can be seen in Fig. 4 in which both traces *A* and *B* were made from the same unit. A small proportion of units appeared to change quite rapidly from a fast firing to a slow firing state in a phasic pattern (see Fig. 4*C*).

It was not possible to predict how an individual neurone in the supraoptic or paraventricular nucleus would respond to intracarotid injections of isotonic and hypertonic sodium chloride. However, a significantly

greater proportion ($P < 0{\cdot}005$; χ^2 test) of the hypertonic injections caused a $> 30\%$ change in the firing rate of supraoptic units than paraventricular units (77/109 injections compared with 54/109). It was also found that isotonic sodium chloride injections frequently altered the firing rate of

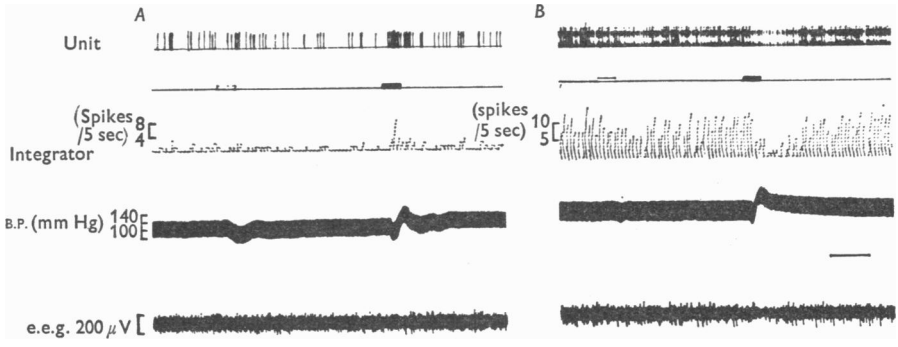

Fig. 3. Records of the changes in firing rate caused by intracarotid injections of isotonic (open rectangles) and hypertonic sodium chloride (filled rectangles) in *A*, a slow firing unit and *B*, a fast firing unit; also shown are blood pressure and e.e.g. traces. One spike is represented by a single pen deflexion on the upper trace and one step on the second trace. The time mark represents 1 min.

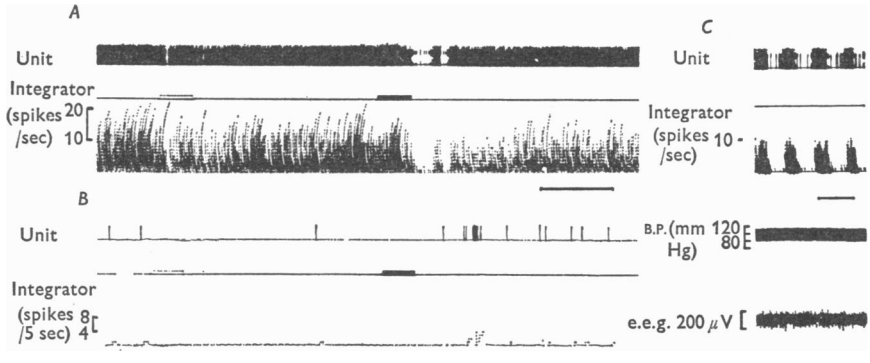

Fig. 4. Records of the changes in firing rate caused by intracarotid injection of isotonic (open rectangles) and hypertonic sodium chloride (filled rectangles) on the firing rate of a single unit; *A*, when it was firing fast and *B*, when it was firing slowly. *C*, a record from a unit which fired in a phasic pattern. In each case the time marker represents 1 min and each spike is presented by 1 pen deflexion on the upper trace and one step on the second trace.

supraoptic units but they did so significantly less often ($P < 0{\cdot}001$; χ^2 test) than did hypertonic injections (47/113 injections compared with 77/109).

Fig. 5 shows a detailed picture of these results. The proportion of

responses of slow firing (< 2 spikes/sec) and fast firing (> 2 spikes/sec) units in both supraoptic and paraventricular nuclei affected by isotonic and hypertonic injections can be seen. Clearly the most obvious change was excitation of slow firing units in the supraoptic nucleus by hypertonic injections. In addition a substantial number of fast firing units in the supraoptic nucleus were inhibited by hypertonic injections. In both cases

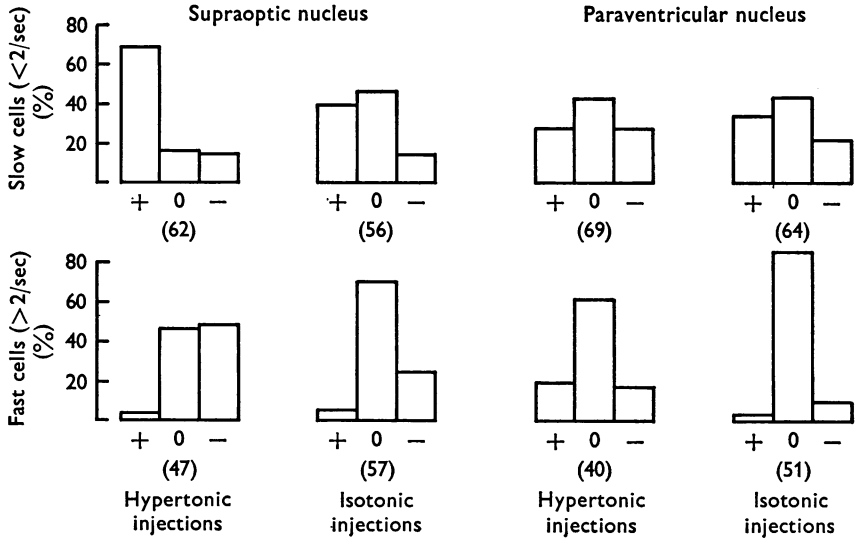

Fig. 5. The proportions of responses of fast and slow firing units in the supraoptic and paraventricular nuclei which were excitatory (+), inconclusive (0) or inhibitory (−). The figures in brackets under each block represent the numbers of responses constituting that block.

significantly more responses occurred after hypertonic than isotonic injections ($P < 0{\cdot}005$ for the slow units and $P < 0{\cdot}01$ for the fast units; χ^2 test).

Fig. 6 shows the time scale of these responses. Consistent with the results shown in Fig. 5 it can be seen that the most obvious changes in mean firing rates were an excitation of slow firing and an inhibition of fast firing supraoptic units. No significant change in mean firing rate occurred more than 2 min after the end of the injections. The change in firing rate of the 'slow' supraoptic neurones following hypertonic injections was significantly greater ($P < 0{\cdot}05$) than that after isotonic injections for eight 15 sec periods and that of the fast supraoptic, for four 15 sec periods. The change in firing rate of paraventricular neurones after hypertonic injections was never significantly greater than that after isotonic injections.

Changes in blood hormone concentration

Fig. 7 shows that, under urethane anaesthesia and after the stress of the surgical procedure outlined in the methods section, a considerable quantity of antidiuretic and milk-ejection activity could be detected in plasma. In addition, after intracarotid injection of hypertonic sodium chloride solution

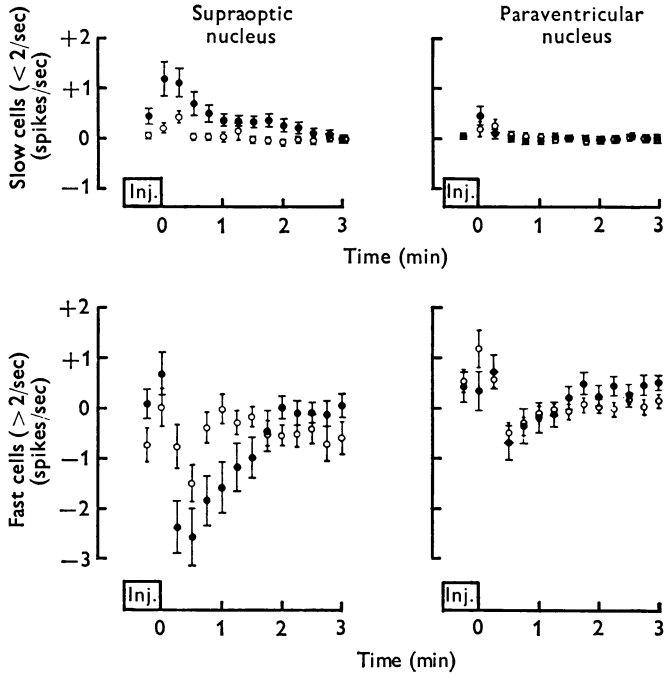

Fig. 6. The mean change in firing rate of fast and slow firing units in the supraoptic and paraventricular nuclei following isotonic injections (open circles) and hypertonic injections (filled circles) of sodium chloride.

an increase in the plasma concentrations of both antidiuretic and milk-ejection activity was detected. There appears to be a slight delay before an increase can be detected in the jugular blood but at 1, 3 and 5 min after injection a substantial increase in both types of activity is seen. At 3 and 5 min for antidiuretic activity and 1, 3 and 5 min for milk-ejection activity the 95% confidence limits of the estimates after hypertonic injection did not overlap either with those of the before-injection estimate or with those of the after-isotonic-injection estimate.

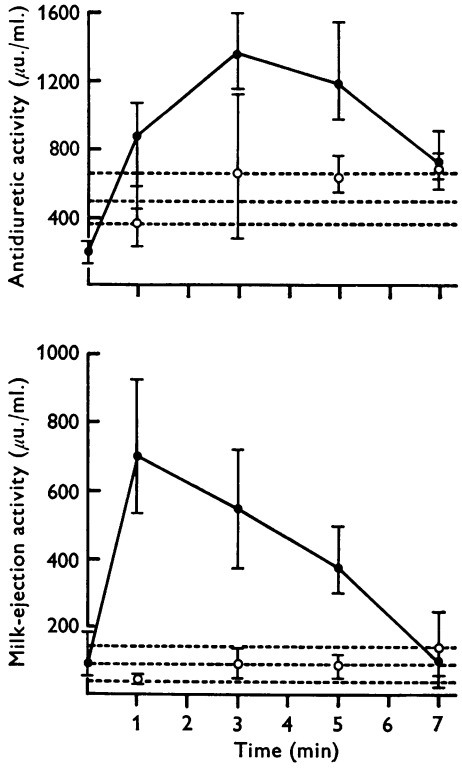

Fig. 7. The changes in concentration of antidiuretic and milk-ejection activity in the pooled plasma samples (vasopressin and oxytocin expressed in terms of μu./ml.) caused by intracarotid injection of hypertonic (filled circles) and isotonic sodium chloride (open circles). The bars indicate the 95 % confidence limits of the estimates. The dashed lines represent the mean concentration before injection together with the means of the upper and lower 95 % confidence limits before injection.

DISCUSSION

There is no doubt that action potentials can be recorded from the cell bodies of neurones within the supraoptic and paraventricular nuclei from which axones lead into the neurohypophysis. These neurones are probably neurosecretory although there is no way of excluding the possibility that they are ordinary nerve cells associated with the neurosecretory neurones. It is widely believed that an increased rate of firing in these units is associated with an enhanced rate of secretion of oxytocin and vasopressin. This implies a simple and direct relationship so that a given number of action potentials will lead to the release of a constant amount of hormone. A significant change in the action potential activity of supraoptic neurones

certainly occurs during secretion but the results of this investigation are not entirely consistent with this simple hypothesis.

Firstly it is difficult to explain the substantial number of inhibitory responses which occurred in fast firing supraoptic neurones following hypertonic injections. These have not been observed by all workers in the field but the finding cannot be regarded as exceptional since Cross & Green (1959) observed several such inhibitory responses. They can only be interpreted, without abandoning the concept of a simple relationship between action potentials and hormone release, by postulating the existence in rats of an inhibitory recurrent collateral system as suggested by Kandel (1964) for goldfish neurosecretory neurones. Excitation of certain neurosecretory neurones might then be expected to inhibit others. There is no direct evidence for this but the observation of individual neurones which reverse their firing characteristics (see Fig. 4*A*, *B*) may imply a periodic inhibition of a proportion of neurosecretory cells. The firing pattern seen in Fig. 4*C* might also be explicable in this way.

Secondly there was some discrepancy between the time scale of hormone release and the change in discharge rate of the units. The greatest alteration in discharge rate occurred immediately after the end of the stimulus and no significant change in firing rate was observed after 2 min. Except for one report at very low plasma concentrations the published half-life for vasopressin is not greater than 2 min (Ginsburg, 1968) and that of oxytocin is 2·1 min (Fabian, Forsling, Jones & Lee, 1969). Preliminary experiments carried out under the condition of this investigation showed that 3 min after a rapid intravenous injection the blood concentration of oxytocin had fallen to 35% of its level immediately after injection and that of vasopressin to 33%. This means that the half-life of oxytocin and vasopressin in blood was not substantially altered by the urethane anaesthesia. Accordingly, if the maximum rate of hormone secretion was associated with the greatest change in unit firing rate and if it had returned to base line when firing rate did so, the blood concentration at 5 min would be expected to be approximately one-half of that at 3 min. This might be said to be true for oxytocin since at 5 min the blood concentration of milk-ejection activity was reduced to 70% of its value at 3 min. In addition, if the milk-ejection potency of vasopressin is taken into account (10·9% with 95% confidence limits of 15·6–7·1% in the assay system used by the author) this value is reduced to 64%. However, it is certainly not true for vasopressin, particularly if the time taken to collect each 1 ml. blood sample is considered. This took approximately 30 sec so that the 2 min blood samples contain blood taken between 2 and 2·5 min after the stimulus. It is likely therefore, unless a substantial quantity of hormone finds its way into extracellular spaces during secretory activity and sub-

sequently leaks into the blood, that the neurosecretory neurones release some additional hormone after the subsidence of the electrical response.

Thirdly the concentration of oxytocin in the initial plasma samples was certainly less than one-fifth of the concentration of ADH in μu./ml., but there was no significant difference between the firing rates of units in the supraoptic and paraventricular nuclei. If the supraoptic nucleus is more concerned with vasopressin release and the paraventricular nucleus with oxytocin (see Bisset *et al.* 1967) and since the half-life of each hormone is similar (Ginsburg, 1968; Fabian *et al.* 1969) a greater difference would have been expected unless the same number of action potentials represents different quantities of vasopressin and oxytocin. Similarly although significantly less paraventricular units than supraoptic units responded to hypertonic injections there was a far greater percentage change in the blood concentration of oxytocin than vasopressin (which is even more obvious if allowance is made for the milk-ejection potency of vasopressin). The latter observation could, however, be explained if the afferent input to the paraventricular nucleus does not have a substantial osmosensitive component and if the supraoptic nucleus has a sufficient oxytocin secreting capacity.

It must be concluded therefore that although there is some correspondence between hormone release and action potential activity, the relationship may not be as simple and direct as the release of neurotransmitter substances appears to be. It is possible that a train of impulses reaching the secretory terminal of a neurosecretory neurone may alter the integrity of the cell membrane, possibly by depolarizing it (Ishida, 1970), so that hormone continues to be released after the end of the impulse train. It is also possible that the action potentials reflect in some way the biosynthetic or storage function of these cells and so only indirectly affect hormone release.

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