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RECURRENT INHIBITION OF ANTIDROMICALLY IDENTIFIED RAT SUPRAOPTIC NEURONES

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SUMMARY

1. In anaesthetized male rats, the hypothalamus and pituitary stalk were exposed by a transpharyngeal approach. The compound field potential of the supraoptic nucleus evoked by stimulation of the pituitary stalk, was recorded with glass electrodes inserted near the origin of the anterior cerebral artery.

2. The mean latency of 169 antidromically evoked action potentials isolated from the field was 9·9 msec with an extreme range of 6–26 msec. Although the wave form of the antidromic action potential showed a variety of shapes and sizes and the initial wave could be of either polarity, the majority were strikingly similar in form. The initial wave was positive with an inflexion on the rising phase and was followed by a shallow rather longer lasting negative potential.

3. The antidromic nature of the action potential was confirmed when the action potential evoked at constant latency after the stimulus was observed to be cancelled by another occurring spontaneously. Although the antidromic action potentials followed stimulation frequencies greater than 100 Hz, the response to high frequency stimulation was seldom tested since the amplitude of the action potential was greatly reduced at frequencies above 30 Hz if the number of shocks exceeded a critical number, as few as 3–6 at 100 Hz.

4. Stimulation of the pituitary stalk at intensities below and near threshold for antidromic invasion of the cell under study was shown by means of post-stimulus time histograms to be associated with an inhibitory period lasting on average 80 msec (S.D. = 13, N = 30).

5. An increase in the intensity and duration of the inhibitory period occurred as the intensity of the stimulation was increased as might be expected if the response was mediated synaptically. The inhibitory path-

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way is believed to involve the recurrent collateral axons already demonstrated anatomically since the stimulation intensities necessary to produce either a marked inhibitory response or antidromic invasion of the cell in question are in most instances nearly the same.

INTRODUCTION

Extensive anatomical and functional investigations have clearly established the dual neural and endocrine nature of the supraoptic and paraventricular nuclei of the hypothalamus and their outflow, the hypothalamo-neurohypophysial tract (cf. Haymaker, Anderson & Nauta, 1969).

The release of vasopressin and oxytocin appears to be quantitatively related to the number and frequency of propagated action potentials elicited by electrical stimulation of the hypothalamo-neurohypophysial tract both *in vivo* (Harris, Manabe & Ruf, 1969) and *in vitro* (Ishida, 1970; Dreifuss, Kalnins, Kelly & Ruf, 1971). Identification of extracellular records from the hypothalamus as belonging to cells of these nuclei by recording action potentials evoked by antidromic stimulation has only recently become an established technique (Dyball & Koizumi, 1969; Novin, Sundsten & Cross, 1970; Yamashita, Koizumi & Brooks, 1970; Barker, Crayton & Nicoll, 1971; Dyball, 1971), although a clear demonstration of the ability of these cells to propagate action potentials was made earlier by Yagi, Azuma & Matsuda (1966).

The action potentials responsible for the release of neurohormones from the nerve endings of the neurohypophysis appear to be no different from those of other neurones of the same shape and size and their frequency is probably modulated by synaptic events on the soma and dendrites of the supraoptic and paraventricular neurones. Intracellular records from the equivalent neuroendocrine cells of the goldfish preoptic nucleus by Kandel (1964) showed that these cells developed action potentials in response to both orthodromic and antidromic stimulation and generated a short latency inhibitory response to electrical stimulation of the pituitary.

Inhibition evoked by stimulation of the pituitary stalk raises the possibility that the axons of goldfish preoptic cells end not only on the capillaries of the posterior pituitary but also, by means of the recurrent collaterals of the preoptic-neurohypophysial fibres, formed synaptic contacts with other nerve cells.

Since the presence of axon collaterals of the mammalian hypothalamo-neurohypophysial tract have been described (Christ, 1966), electrophysiological evidence for antidromic inhibition of mammalian neuroendocrine cells was sought. Some preliminary findings have been reported (Kelly & Dreifuss, 1970).

METHODS

Experiments were performed on twenty-two male albino rats of a Carworth strain, 350–450 g body weight. Initial anaesthesia by an intraperitonal injection of a mixture of urethane and sodium pentobarbitone (400 and 50 mg/kg respectively) was supplemented by 5 mg doses of sodium pentobarbitone injected intravenously as required every 2–3 hr. Animals were fixed supine by a simple surgical headholder. The trachea, the cephalic end of the contralateral common carotid artery and a branch of the jugular vein were cannulated. The base of the skull was approached by dividing the soft palate and retracting the hemisected mandibles laterally and the partially freed tongue caudally. Under the binocular microscope, the base of the brain was exposed by removing the sphenoid bone with a dental drill. Bleeding was controlled by packing the venous sinuses with oxy-cellulose. On the right side, the aperture was extended to expose anteriorly the optic chiasma, posteriorly the pituitary fossa and laterally the optic tract as it passes below the origin of the middle cerebral artery.

Stimulation of the pituitary stalk was by means of bipolar silver electrodes placed across the exposed pituitary stalk viewed through the microscope.

Extracellular recording was from a 3 M-NaCl-filled channel of double or multi-barrelled micropipettes with tip resistance of 10–20 MΩ. Each electrode track was started with the electrode tip placed on the optic tract exposed through openings torn in the overlying pia, just lateral to the common carotid and anterior cerebral arteries on either side of the origin of the middle cerebral artery. The entire wound was sealed with 5% Agar/saline poured around the recording and stimulating electrodes to prevent drying and to limit vascular pulsations of the brain. The micro-electrode was connected to a 3A9 amplifier (selected bandpass 0·1–10 KHz) of a Tektronix 565 oscilloscope by means of a wide band electrometer (W-P Instruments, Inc.).

Individual action potentials were converted by an adjustable voltage gate into standard pulses monitored by an audio-amplifier and used to increase the *Z*-axis intensification of the oscilloscope in order to brighten the selected spikes with respect to the background activity. The output pulses of the gate were also available for data reduction by either a resettable counter connected to a strip chart recorder or a special purpose computer (Biomac 1000).

RESULTS

Compound field potential

Compound field potentials evoked by antidromic shocks applied to the exposed pituitary stalk, were recorded by a micropipette inserted just below the exposed ventral surface of the hypothalamus in the region of the bifurcation of the internal carotid artery. Consistent results similar to those shown in Fig. 1 were obtained when the micropipette was inserted lateral to the origin of the anterior cerebral artery close to the visible boundary between the median eminence and the optic tract. Rarely was it necessary to insert the micro-electrode more than a few hundred microns before locating the field potential. Seldom was the field found to extend beyond a depth of 500 μ. In a number of experiments where the electrode was located more posteriorly on the optic tract, lateral and posterior to the origin of the middle cerebral artery, the compound field potential proved

more difficult to locate and it was necessary to insert the electrode tip approximately 500 μ deep to the pial surface.

The antidromic compound field potential evoked by shocks 3–20 V intensity 0·5 msec duration, had a latency of approximately 8 msec and lasted rather longer than 10 msec. Characteristically it was made up of

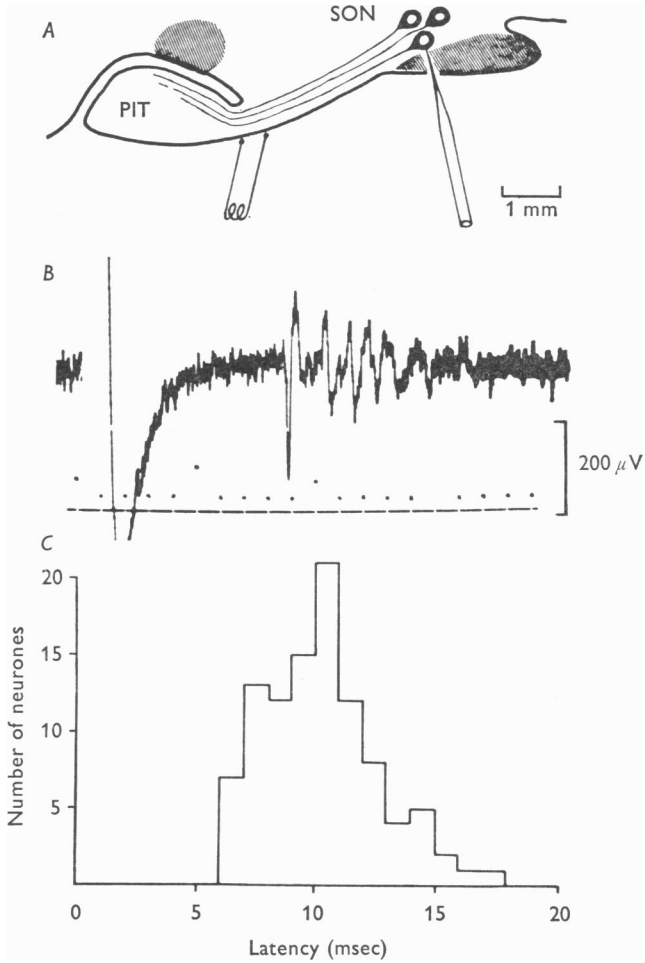

Fig. 1. *A*, schematic representation of the bipolar stimulating electrode position on the pituitary (PIT) stalk and the micropipette penetration of the ventral hypothalamus near the supraoptic nucleus (SON); *B*, extracellular recording of a typical compound field potential evoked by a 9 V shock to the pituitary stalk; upward deflexions in this and subsequent records are positive, time calibration 1 msec; *C*, histogram of the observed latency of antidromic action potentials recorded from ninety-nine cells whose mean latency was 9·5 msec.

a series of 'spike-like' components one or more of which not infrequently showed the all or none phenomena as the intensity of stimulation was altered.

The antidromic action potential

By isolating all-or-none action potentials from this field potential it proved possible to identify 169 cells of the supraoptic nucleus. The mean latency of the antidromic action potential was 9·9 msec (S.D. = 6·5) with an extreme range of 6–26 msec (Fig. 1). The conduction velocity appeared to be less than 1 m/sec as has been described by other authors, and is consistent with the small diameter of the non-myelinated nerve fibres.

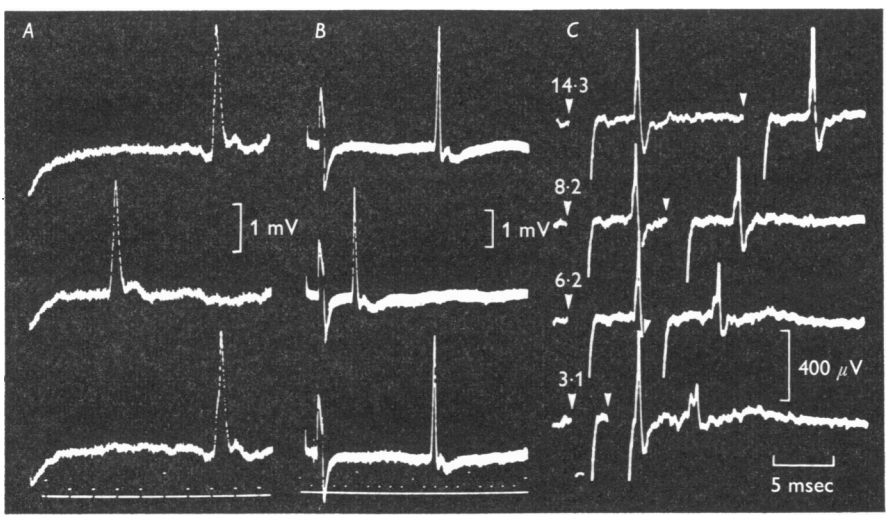

Fig. 2. Antidromically evoked action potentials. *A* and *B*, control records show a spontaneous action potential responsible for cancellation of the antidromic action potentials and a characteristic notch is seen on the positive rising phase of the action potential; *C*, two shock study in which the second shock artifact is marked by an arrow and the numbers near the origin of each trace show the inter-shock interval in msec; time calibrations in *A* and *B*, 1 msec.

Several features of our records suggested that the action potentials were recorded from the vicinity of cell bodies or basal dendrites rather than the axons of the tract. Unitary spikes were only isolated from the field in the anatomical region of the nucleus. Action potentials were seen to change from negative-positive potentials to larger predominantly positive spikes as the electrode was advanced. Although spike activity of single cells could only be isolated from that of their neighbours with fine-tipped electrodes, stable recordings once established often persisted for several hours. Positive

spikes with an inflexion of the rising phase, can be recorded from axons (cf. Bishop, Burke & Davis, 1962*a*); however they are of short duration and rather unstable, characteristically appearing and disappearing without warning.

Although the wave form of the antidromic action potential showed a great variety of shapes and sizes and the initial wave could be of either polarity, the majority of fully developed spikes were strikingly similar in form. The initial wave was positive with an inflexion in the rising phase and was followed by a shallow, often notched, longer lasting 'late negative wave phase'.

The inflexion in the rising phase became more prominent as the frequency of stimulation was increased. Occasionally, in studies where two successive stimuli were applied to the pituitary stalk, it was possible to demonstrate dissociation of the antidromic spike into two components, indicating failure of invasion into a region with a lower safety factor (presumably from the initial segment (I S) into the soma and dendrites (SD)).

The action potential evoked by a second stimulus of equal intensity could be reduced as shown in Fig. 2*C*, to little more than an I S spike by shortening the inter-shock interval to less than 4 msec. Any further reduction in the interval between shocks led to complete block of the second spike. No reduction in the amplitude of the second spike could be detected when the inter-shock interval exceeded 14·3 msec. In another cell studied in detail a minimal change in the amplitude of the second spike occurred when the inter-shock interval was reduced below 16·0 msec and the absolute refractory period was 5·8 msec.

Both the shape and conduction velocity of the majority of the antidromic action potentials were therefore strikingly similar to those recorded from the paraventricular nucleus of the lactating rabbit and examined in great detail by Novin *et al.* (1970) and from the supraoptic and paraventricular nucleus of the cat by Yamashita *et al.* (1970). Stable large positive spikes with prominent inflexions of the rising phase related to I S, SD phenomena are characteristics of recordings from small tipped electrodes located near the cell soma or origin of the dendritic processes (Bishop *et al.* 1962*b*). The shape of the 'late negative phase' may also be a function of the location of the electrode tips, and the failure of the impulse to invade part of the dendritic tree, rather than a special feature of endocrine cells as suggested by Novin *et al.* (1970).

Antidromically evoked impulses could usually be distinguished from those occurring spontaneously by their constant latency. The most useful confirmatory method proved to be the cancellation of the antidromic spike by collision with an orthodromic spontaneously occurring spike as shown in Figs. 2 and 4. Tests to determine the ability of antidromic action

potentials to follow high frequency stimulation were avoided since tetanic stimulation caused both a reduction in the amplitude of the action potential (Fig. 3) and a depression of spontaneous activity (Fig. 4). When the

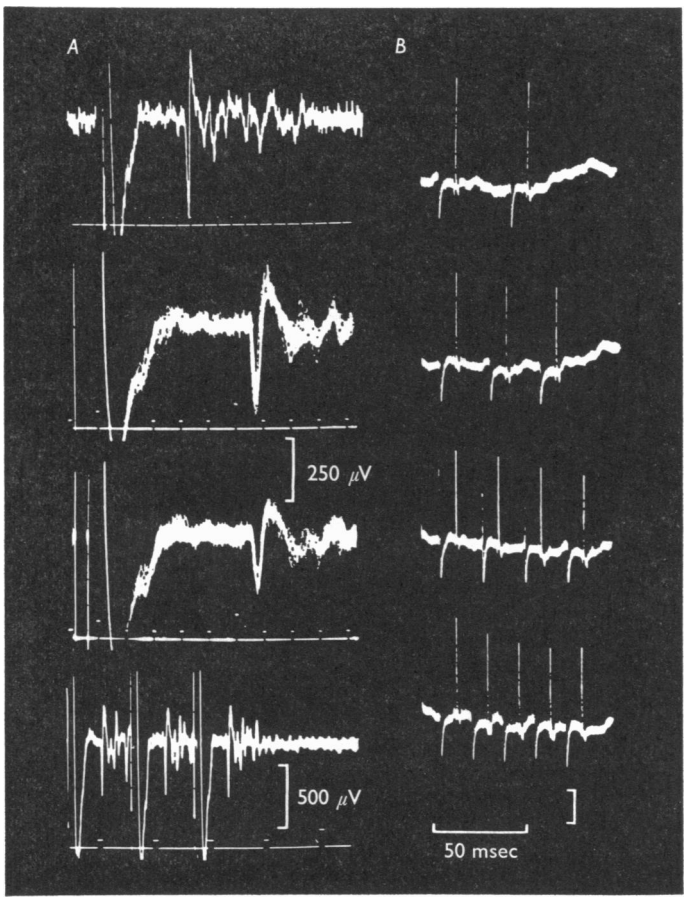

Fig. 3. The influence of stimulation frequency on the amplitude of the antidromic action potential. *A*, control record is uppermost and the two subsequent records show about ten superimposed records photographed at 0·5 and 1·0 sec after the onset of stimulation at 100 Hz. The lowest record, at 0·5 × gain shows a three shock train delivered at about the same frequency and repeated every 2·0 sec; time calibration for the upper three traces and the lowest trace 1 and 10 msec respectively. *B*, records from another cell to show the effect of short trains delivered at 2·0 sec intervals.

stimulus intensity was increased several fold above the threshold for the axon of the cell under study, electrical stimulation of the pituitary stalk evoked only *one* action potential per stimulus. With an orthodromic path-

way, increases in the stimulus intensity would be expected to lead to a progressive increase in the amplitude of post-synaptic potentials resulting in a decreased latency and perhaps multiple discharge.

High frequency stimulation

The amplitude of the antidromic action potential was extremely sensitive to stimulation frequencies greater than about 30 Hz. When the stimulation frequency exceeded a critical value for a particular cell, a rapid reduction in the action potential amplitude occurred. The critical value was extremely variable from cell to cell and proved impossible to characterize in a systematic manner. The amplitude of the action potentials of the cell shown in Fig. 3*A* was unaltered during a stimulus train whose frequency approached 100 Hz provided the number of shocks within the train was less than 6 and the train was repeated only once a second. When the train duration was increased, however, the spike amplitude was reduced by 50% of its initial value after about 100 shocks had been presented. In the other cell shown in Fig. 3*B*, as few as 2 or 3 shocks repeated every 2 sec were sufficient to reduce the action potential amplitude when the frequency was higher than 50 Hz.

The reduction in amplitude of the action potential was probably not the result of the inactivation of the sodium-carrier mechanism as seen during the relative refractory period, since fractionation of the spike into a pure I S component was rarely achieved by high frequency stimulation. The change in amplitude was probably related to a reduction in the transmembrane potential. A reduction in spike amplitude associated with a depolarization during tetanic stimulation appears to be a constant feature of peripheral mammalian C fibres with conduction velocities similar to those found for this pathway (Brown & Holmes, 1956; Ritchie & Straub, 1956). Presumably the cell bodies of small unmyelinated fibres might also be subject to progressive depolarization during tetanic stimulation as a result of sodium accumulation. During repetitive activity the influx of sodium during the action potential may be responsible for both the facilitation of hormone release which occurs at stimulation frequencies between 10 and 35 Hz and the decline at higher frequencies observed in studies of the neurohypophysis both *in vivo* by Harris *et al.* (1969) and *in vitro* by Dreifuss *et al.* (1971). Sodium accumulation during tetanic stimulation appears to cause facilitation of transmitter release followed by complete transmission failure at the frog neuromuscular junction (Birks & Cohen, 1968).

Spontaneous action potential

More than 80% of the cells identified by antidromic action potentials were spontaneously active. In shape, these action potentials closely resembled the antidromic action potential. If the rising phase of the antidromic action potential of a particular cell were notched, the spontaneous action potential not uncommonly also showed some inflexion of the rising phase although perhaps less marked. In the same cell, the shape and ampli-

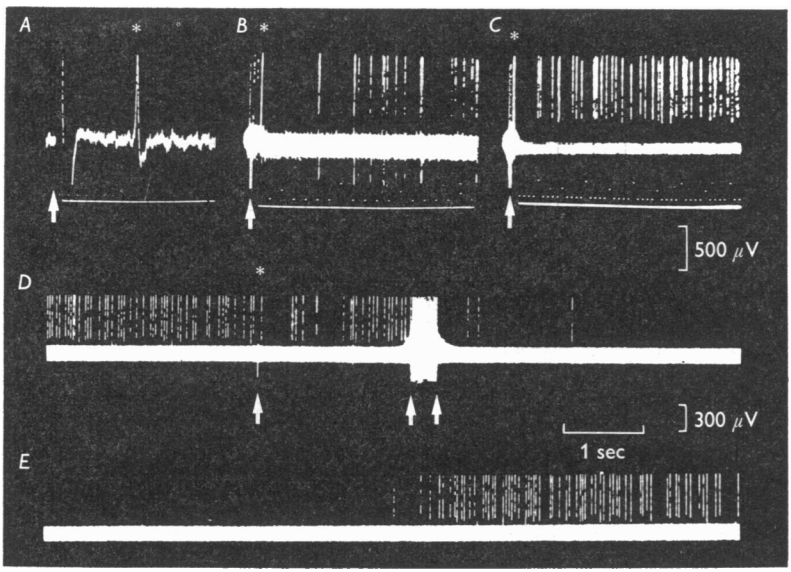

Fig. 4. Depression of the spontaneous discharge by antidromic stimulation. *A*, control antidromic action potential at a fixed latency of 9·5 msec. *B* and *C*, ten oscilloscope traces superimposed photographically to show suppression of the spontaneous firing by stimulation of the pituitary stalk. *D* and *E* are contiguous records from moving film to show the recovery of the spontaneous discharge following a single and a train of shocks at 100 Hz marked by single and double arrows respectively. Stimulus artifacts are marked by arrows; antidromic action potentials by *, and time calibrations are 1 msec for *A* and 10 msec for *B* and *C*.

tude of the 'late negative phase' of the spontaneous spikes (Fig. 2*A*, *B*) was in most instances indistinguishable from that of the action potential evoked antidromically. Often when compared with the antidromically evoked spike, the amplitude of the spontaneous action potential was found to be 10–30% smaller. The reduction in amplitude is presumably a reflexion of the persistence of the dendritic depolarization responsible for initiating the spontaneous spike; a smaller spike would result from

a reduction in the voltage difference between the partially depolarized dendrite and the cell soma (cf. Rosenthal, 1967).

Antidromic inhibition

High frequency stimulation of the pituitary stalk not only modified the amplitude of the antidromically evoked action potential as described earlier, but also led to a long-lasting depression of the spontaneous spike discharge. Continuous records *G* and *H* in Fig. 4 are from a cell identified as belonging to the supraoptic nucleus by cancellation of the antidromically evoked action potential by a spontaneous action potential. The spon-

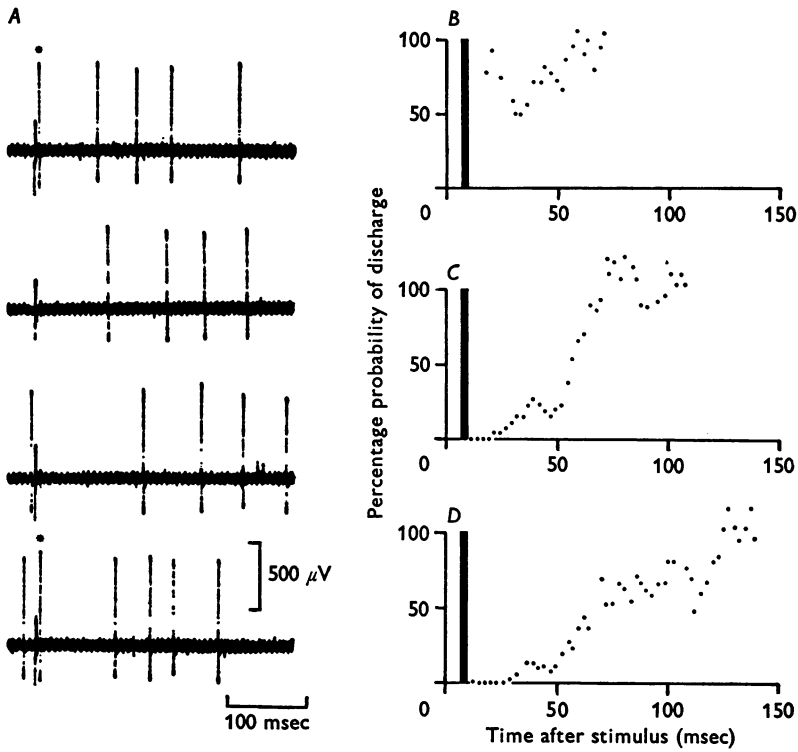

Fig. 5. Duration and intensity of the reduction in the probability of discharge. *A*, single oscilloscope sweeps show the depression of the spontaneous discharge by pituitary stalk stimulation in the presence and absence of the antidromic action potential marked by *; *B–D*, smoothed post-stimulus time histograms from an identified cell to show the effect of 2·5 V (*B*), 3·0 V (*C*) and 12·5 V (*D*) stimulation. Threshold = 3·0 V. The histograms were smoothed by averaging five adjacent addresses (2·56 msec) and expressing this mean as a percentage of the random firing frequency from control histograms computed in the absence of stimulation. Antidromic action potentials were counted into the bins identified by closed bars. The stimulus frequency was 1·7 Hz and 250 stimuli were counted.

taneous discharge was almost abolished by a train of shocks applied to the pituitary stalk at a frequency of 100 Hz. Whereas the antidromic action potential evoked by a near threshold single shock of 6·0 V was followed by a period of silence and reduced excitability lasting less than a second, the high frequency train at 100 Hz and the same intensity caused an almost complete cessation of spontaneous activity which lasted nearly 6 sec.

It was possible in the majority of cells, as shown in Fig. 5*A*, to demonstrate a silent period following stimulation of the pituitary stalk in the *absence of an antidromic action potential* by photographing randomly single oscilloscope sweeps with the stimulus intensity just below threshold for 50% antidromic invasion of the cell. Since the period of reduced probability of discharge occurs in the absence of stimulus-coupled excitation the synaptic nature of the inhibition seems undisputable. In addition both the intensity and duration of the inhibition are related to the stimulus strength once threshold has been attained. The contribution of the antidromic action potential and associated post-spike depression to the inhibitory period must be small since two shock studies described earlier failed to show a decrease in the membrane excitability at inter-shock intervals greater than 16 msec.

As shown in Fig. 4*B*, *C*, superimposition of ten oscilloscope traces photographically enhanced the demonstration of the silent period and the reduction in the spontaneous discharge which followed a single shock to the pituitary stalk. Every identified cell tested with single shocks of just sufficient intensity to activate the cell antidromically showed inhibition of sufficient intensity and duration to be recognized by this simple technique. In thirty cells in which the stimulus intensity ranged from 2 to 20 V the mean inhibitory period was approximately 80 ± 13 msec (S.D. of an observation).

In seventeen of these cells, a more complete quantitative analysis has been made by computing estimates of the probability that the unit will fire an impulse at various times after the stimulus. Post-stimulus time histograms obtained with a Biomac 1000 special purpose computer are illustrated in Fig. 5.

The sensitivity of the period of reduced probability of discharge to an increase in the stimulus strength is shown in Fig. 5*B*, *D*, where three smoothed histograms from another identified cell are presented. When the stimulus straddled threshold (2·5 V, 26% effective) an obvious reduction in the probability of discharge occurred during the first 64 msec after the stimulus. However, an increase in the stimulus intensity of only 0·5 V led to a dramatic decrease in the probability of discharge during the same period. Increasing the stimulus intensity to 12·5 V caused the period of reduced probability of firing to be more than doubled in duration.

Determination of the minimal latency for the onset of the inhibitory response proved impossible from the majority of post-stimulus histograms computed from identified supraoptic-nuclear cells, since an increase in the strength of stimulation led to antidromic excitation. Following the antidromic potential there was almost invariably a complete absence of spikes from the bins of the histogram and it was not possible to detect the transition from post-spike depression to synaptic inhibition. However, in the

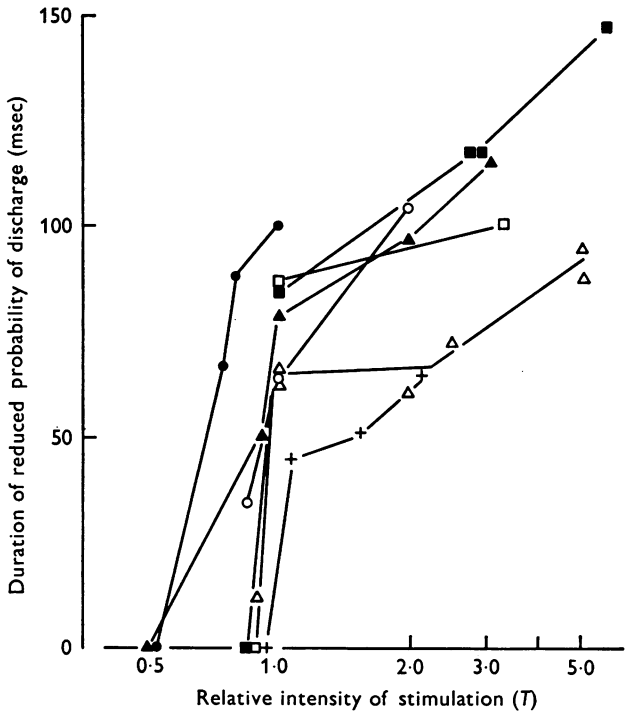

Fig. 6. The relationship between the duration of the reduction in the discharge probability evoked by antidromic stimulation and the stimulus intensity expressed in terms of T, the threshold for antidromic invasion of the cell under study, plotted on a $\log_{10}$ scale.

histogram shown in Fig. 5*B* where the stimulus strength was straddling threshold and the antidromic spike was absent from 73% of the sweeps, the latency of the onset of the inhibition began approximately 5 msec after the antidromic spike. Although an increased delay of 5 msec would allow for at least one synapse, the great dispersion between the fastest and slowest conduction velocities of the supraoptic axons (Fig. 1) makes the exclusion of a direct inhibitory pathway impossible.

In Fig. 6 the duration of the inhibition observed in experiments on seven

different cells is compared with the stimulus strength, expressed in terms of threshold plotted on a $\log_{10}$ scale. Consistent with the suggestion that the inhibitory response is mediated synaptically, the duration of the inhibitory response was approximately linearly related to the stimulus intensity provided the stimulus was of sufficient intensity to ensure antidromic invasion of the cell under study. At and below threshold for initiation of an antidromic action potential, however, the relationship became extremely steep and no inhibition occurred when the stimulus intensity was 0·7 of threshold. The great similarity between the antidromic and inhibitory thresholds supports the hypothesis that the inhibition is a consequence of antidromic invasion of supraoptic cell axons projecting into the pituitary stalk (although not necessarily the axon of projection of the particular cell inhibited) rather than the result of current spread to nearby structures. Indeed, the inhibitory pathways converging on each cell may be restricted to only a few axons whose current requirement for excitation is comparable. Since the pituitary stalk consists of a bundle of fibres of approximately equal diameter, the number of additional fibres excited by a small increment in the stimulus intensity may simply reflect their close topographical proximity to each other within the stalk rather than any more complex characteristic.

Further support for the view that the inhibitory effect is restricted to neurones whose axons can be excited antidromically was the finding that only three of more than nineteen spontaneously active cells found very close to the antidromically identified cells, but not invaded by antidromic action potentials during pituitary stalk stimulation, could be shown to be inhibited. Two of these cells may have been neurosecretory cells whose axons were inexcitable since they were the only two cells of this group to have action potentials whose initial wave form was positive and showed the characteristic inflexion on the rising phase described earlier and whose spontaneous discharge and response to acetylcholine applied by iontophoresis resembled that of identified cells described in a companion paper (Dreifuss & Kelly, 1971).

DISCUSSION

In their original study of single units of the hypothalamus Cross & Green (1959) found antidromic stimulation of the neural lobe an unsuitable means of identifying cells of the supraoptic nucleus. More recent studies, however, have been greatly facilitated by this approach in both the rat (Yagi *et al.* 1966; Dyball & Koizumi, 1969; Dyball, 1971) and the cat (Yamashita *et al.* 1970; Barker *et al.* 1971). In the present study spontaneously active neurones were positively identified as belonging to the supraoptic nucleus when action potentials evoked by stimulation of their

projecting axons in the pituitary stalk were seen to be occluded by a spontaneously occurring action potential. In all of the cells identified in this way the antidromically evoked action potential was followed by a period of reduced probability of firing which proved indistinguishable from recurrent inhibition seen elsewhere in the central nervous system when the appropriate tests were applied (cf. Gordon & Jukes, 1964). More specifically, inhibition resulted from antidromic shocks subthreshold for the test axon and cannot, therefore, be the result of excitability changes of the cell induced by invasion by an antidromic spike and must be attributed to the activity of adjacent axons excited by the shock to the pituitary stalk.

The inhibitory period could result from a direct collateral pathway with temporal dispersion of the presynaptic volleys converging on a particular supraoptic neurone. The extremely large difference in conduction velocity, equivalent to a latency difference of 20 msec between the fastest and slowest fibres, makes this a real but unprecedented possibility. However, an inhibitory period of approximately 80 msec in duration evoked by stimulus intensities near threshold for the test axon yet well below threshold for invasion of the majority of cells by an antidromic action potential, can best be attributed to interneurones interpolated between the terminals of the collaterals and the supraoptic nucleus.

By analogy with the Renshaw inhibition of spinal motoneurones (Eccles, Fatt & Koketsu, 1954), these fibres may be presumed to have collateral branches which act in a recurrent fashion on the nucleus either directly or through one or more interneurones. Similar inhibitory effects have been demonstrated by stimulating the projecting axons of Betz cells of the cerebral cortex (Phillips, 1959), pyramidal cells of the hippocampus (Kandel, Spencer & Brinley, 1961), the olfactory mitral cells (Phillips, Powell & Shepherd, 1963), the Mauthner neurones of the goldfish (Furukawa & Furshpan, 1963), the cuneothalamic relay cells (Gordon & Jukes, 1964), the thalamo-cortical relay cells of the somatic sensory thalamus (Anderson, Brooks, Eccles & Sears, 1964), principal cells of the lateral geniculate (Burke & Sefton, 1966) and the sacral parasympathetic neurones (De Groat & Ryall, 1968). None of the unidentified cells studied however were found to be excited by shocks to the pituitary stalk. This, however, does not exclude the existence of inhibitory interneurones.

This convergence of the inhibitory pathway on the supraoptic neurones must be more restricted than for example the convergence of the antidromic pathway on pyramidal tract neurones of the cerebral cortex which is very much more obvious. Stefanis & Jasper (1964) found more than 20% of the cells were inhibited by shocks subthreshold for excitation of their own axon. Nevertheless, convergence must occur since increases in the

stimulus over threshold for invasion of the test axon lead to graded increases in both the intensity and duration of the inhibitory period. Inhibition was also restricted in another sense since all but a few of the inhibited cells were identified by antidromic invasion. In much the same way antidromic inhibition of cells in the cuneate nucleus appears to be restricted to cells whose axons project in the medial lemniscus (Gordon & Jukes, 1964).

The inhibitory effects described above yield little information on the inhibitory mechanism. Further analysis can, perhaps, only be obtained from intracellular records. However, the post-stimulus histograms which displayed the inhibitory period evoked by antidromic volleys, recorded in the presence and absence of a discharge evoked by the microelectrophoresis of acetylcholine, were indistinguishable. Since the action of acetylcholine is probably due to an interaction with receptors on the post-synaptic membrane (Dreifuss & Kelly, 1971), the site of inhibition is probably post-synaptic. A similar depression of firing after stimulation of the pituitary has been described by Kandel (1964) in the goldfish preoptic nucleus. By intracellular techniques stimulation of the pituitary was shown to evoke an antidromic inhibitory post-synaptic potential whose amplitude was sensitive to polarizing currents and a reversal potential was demonstrated. The latency differential for antidromic excitation and antidromic inhibition allowed for only one interneurone to be intercalated on the recurrent inhibitory pathway.

Branching of the nerve fibres of the hypothalamo-neurohypophysial tract have been described (Cajal, 1911). In the human, at the level of the infundibulum, fine nerve fibres have been shown to branch off the hypothalamo-neurohypophysial tract (Christ, 1966, Fig. 9). However, the final destiny of these collaterals is unknown, and it is not yet established whether they terminate on blood vessels or on nerve cells. Further evidence for the existence of recurrent collaterals of the axonal outflow of supraoptic and paraventricular neurones derives from degeneration studies in the rat (Olivecrona, 1957), and from studies made on human brains some weeks or months after surgical stalk section at hypophysectomy. Severe cell loss is observed in the supraoptic and paraventricular nuclei, and less advanced changes occur in other infundibular nuclei, which according to Beck & Daniel (1959) are consistent with trans-synaptic rather than retrograde degeneration.

In a companion paper (Dreifuss & Kelly, 1971), evidence is cited which suggests that the inhibitory antidromic pathway is functionally active. Alternatively the recurrent collateral pathway may be the remnants of synapses which lost their importance as the neurosecretory activities of the pathway were developed.

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