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A STUDY OF THE PARAMETERS OF ELECTRICAL STIMULATION OF UNMYELINATED FIBRES IN THE PITUITARY STALK

By G. W. HARRIS, Y. MANABE* and K. B. RUF†

From the Medical Research Council Neuroendocrinology Unit, Department of Human Anatomy, University of Oxford

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SUMMARY

1. The milk-ejection response in lactating rabbits has been used to study the effect of electrical stimuli of different types applied to the supra-opticohypophysial tract in the pituitary stalk.

2. Sine-wave alternating-current pulses were compared with balanced biphasic square-wave pulses of the same frequency and peak-to-peak current strength. At a pulse duration of 2–4 msec the square-wave stimulation was less effective than the sine wave, but at a pulse duration of 8 msec and over, more effective.

3. Above threshold levels of 0·12 mA for the current strength, and of 0·5 msec for the pulse duration, the response increased with increasing current strengths to 2·4 mA and increasing pulse durations to 10 msec.

4. With constant and effective strength and duration of the pulse a slight diminution of the response was seen as the frequency was diminished from 100/sec to 50/sec. Further diminution in the frequency revealed that at some point between 50 and 10 c/s a sudden abrupt diminution in the magnitude of the responses occurred over a small range of frequency. This was a reversible phenomenon and it is suggested that it may be related to neurosecretory events occurring in the nerve terminals.

5. Stimuli with parameters within the ranges mentioned above are effective in eliciting an oxytocic response on the uterus in the rabbit and ovulation responses (when applied to the hypothalamus) in rats and rabbits. It appears likely that stimuli with such parameters are suitable for experiments concerned with stimulation of the hypothalamus.

* Present address: Department of Obstetrics and Gynaecology, University of Kyoto, Japan.

† Present address: Department of Physiology, University of Geneva, Switzerland.

INTRODUCTION

In studies concerned with the function of the hypothalamus the technique of electrical stimulation has been of much importance. Ever since the series of papers by Karplus & Kreidl (1909 and onwards) many workers have applied electrical stimuli to different hypothalamic regions and described a variety of autonomic and endocrine responses. The classic work of Hess (1932) drew attention to the importance of the parameters of the electrical stimulus used in affecting the responses obtained. However, later workers have employed a variety of, in many cases ill-chosen, stimuli. In a recent review of electrical stimulation of the brain Mickle (1961), in referring to the techniques used, states that 'frequency and duration have been chosen largely on the whim of the experimenter' and 'the choice of voltage levels has been based on the investigator's preference rather than on experimentally demonstrated facts'. Only a few systematic studies of stimulation parameters, such as those of Ward (1959) and Lilly (1961), appear to have been made.

In the field of neuroendocrinology it has been shown that electrical stimulation of the hypothalamus will result in the discharge of various anterior pituitary hormones. In an attempt to analyse the mechanism involved, it became desirable to assay the concentration of releasing factors in hypophysial portal vessel blood before and after such stimulation. In order to assess the efficiency of electrical pulses of different parameters, when applied to fine unmyelinated nerve fibres, an investigation has been made of the effects of stimulating the nerve tract controlling posterior pituitary secretion by currents of varying types, since the effects of such currents can be most quickly and repeatedly assessed by measuring the milk-ejection response to oxytocin secretion. The results of this investigation are presented below, and it is felt that in all probability they apply to the unmyelinated fibres concerned with the control of anterior pituitary function. In assessing the results of the present investigation it is useful to remember that the nerve fibres in the supra-opticohypophysial tract in the infundibular stem run as a rich bundle of parallel nerve fibres, that they liberate oxytocic hormone directly from their nerve endings into the blood stream and that this hormone acts directly on the myoepithelial tissue of the mammary gland to evoke ejection of milk (see Fig. 1).

METHODS

Female rabbits, mostly of a Chinchilla breed, of a body wt. 2·5–4·5 kg, were used. Thirty-two animals, and a total of about 600 milk-ejection responses to electrical stimulation, have been studied. The experiments were carried out between the 3rd and 9th day of lactation, the litter being separated from the mother the night before use. Anaesthesia was induced by intravenous injection of 10 % urethane and 2 % chloralose solution (2·5–3·0 ml./kg body wt.), supplemented by ether given by tracheal intubation. The electrodes were inserted into the hypothalamic region by use of a stereotaxic machine and X-ray control, and a teat duct cannulated according to the method of Cross & Harris (1952). The duct cannula was connected to a Grass volumetric pressure transducer (model PT 5 A) and recordings of the milk-ejection responses made on a Grass polygraph (model 5D). The electrodes used were constructed of platinum wire (s.w.g. 34) insulated to within 1 mm of the tip by glass capillary tubing. In twenty animals unipolar stimulation was applied to the nerve fibres in the infundibular stem (indifferent electrode in the rectum), and in twelve rabbits stimulation was carried out by bipolar electrodes, 1·5 mm part, an electrode being situated either side

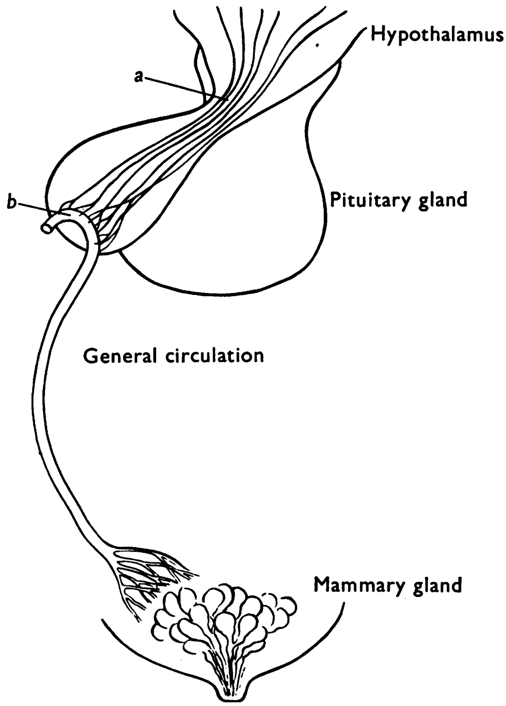

Fig. 1. Diagram of a sagittal section through the pituitary gland and structures concerned in the milk-ejection response. (*a*) Site of stimulation; parallel bundles of the nerve fibres of the SOH tract in the pituitary stalk. (*b*) Site of neurosecretory nerve endings. (*c*) Site of action of oxytocic hormone on the myoepithelial tissue of the mammary gland.

of the infundibular stem. The electrodes were inserted step by step and the effects of a test stimulus (< 0·5 mA) assessed on the milk-ejection response until an optimum site was reached. Other useful criteria of the position of the electrodes were also afforded by X-ray photographs and by observation of the effect of stimulation on the orbital structures. Square-wave stimulation was applied by means of a Nuclear Chicago constant current stimulator (model 7153) and sine-wave stimulation by means of a transformer box and rheostat using the 50 c/s mains supply. In all the experiments in which square-wave pulses were used to stimulate, these were *accurately balanced biphasic pulses* which are easily obtainable with the stimulator used. As added precautions to eliminate any unidirectional d.c. component a sensitive d.c. microammeter (Taylor, range 0–25 μA) was included in the output circuit; and in every case the current flow was continuously monitored by means of an oscilloscope (Solarscope, CD 711, S 2; Solartron, Ditton, Surrey) connected across a 1 kΩ resistor in series with an electrode lead. Figure 2 illustrates the type of wave forms used. For both the sine-wave and square-wave stimulation the train duration was kept constant at 30 sec. In the case of the sine-wave stimulation studies were made of the effect of varying the current strength on the response, and in the case of the square-wave stimulation the current strength, frequency and pulse duration were varied. In view of the fact that repetitive milk-ejection responses to repeated injections of oxytocic hormone or to electrical stimuli

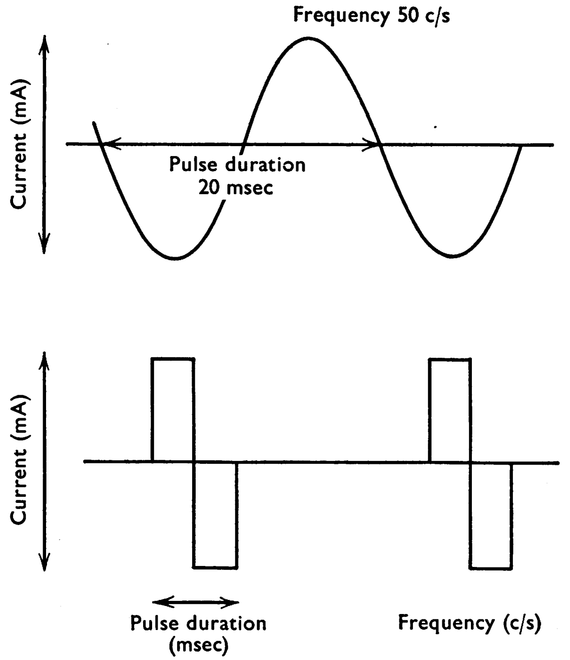

Fig. 2. Diagram to illustrate the wave forms of the currents. Note that the current strength refers to the peak-to-peak flow in mA, and the pulse duration refers to the duration of both phases of the pulse in milliseconds.

may tend to decrease, comparisons between stimuli of different parameters were always made with repeated use of a constant reference stimulus, and a constant interval of 5–6 min allowed between consecutive responses. At the end of each experiment a standard dose of 50 m-u. synthetic oxytocin (Syntocin: Sandoz, Basle) was given intravenously to test the sensitivity of the individual preparations. In a few cases (see below) the electrodes were then permanently fixed to screws in the vault of the skull with dental cement and the animals allowed to recover from the anaesthetic. At the end of the experiments the rabbits were killed, perfused through the abdominal aorta with 10 % formol-saline, and serial sections made through the pituitary region to define the electrode position.

RESULTS

Response to exogenous oxytocin. In order to assess the sensitivity of the milk-ejection response using the pressure transducer and the Grass polygraph recording technique, preliminary experiments were conducted in which exogenous (synthetic) oxytocin was administered by intravenous injection into the marginal vein of the ear. On many occasions administration of 0·5–1·0 m-u. resulted in a clear-cut milk-ejection response. This was most often the case when such a dose was given early in the experiment, but a decrease in sensitivity was frequently observed after multiple responses had been obtained in any one particular animal. In general, the threshold of sensitivity in the present experiments was similar to that reported by other workers (1 m-u., Cross & van Dyke, 1953; 3 m-u., Fitzpatrick & Bentley, 1968). Figure 3*A* illustrates the responses in a rabbit to injections of 1–5 m-u. oxytocin. It may be seen that the log. dose–response curve is approximately linear (Fig. 3*B*), although the latency of the response as measured from the beginning of the injection period is less for the injections made in 10 sec.

Comparison of sine-wave alternating-current pulses with square-wave pulses of different pulse length. A comparison was made of the effectiveness of stimulation of the supraopticohypophysial tract with sine-wave alternating pulses and biphasic square-wave pulses of the same frequency (50 c/s) (as shown in Fig. 2). In experiments on any individual animal in this group the peak-to-peak current flow was always the same for the two types of stimulation (usually 0·5–1·5 mA). The variable factor studied was the duration of the biphasic square-wave pulse (varied from a 2 msec pulse duration to 10 msec). The results obtained on ten animals show that for a frequency of 50 c/s and a given current strength, the milk-ejection response to the square-wave stimulation increases considerably as the duration of the pulse is increased from 2 to 10 msec (see Fig. 4). The responses to the shorter square-wave pulses (2–4 msec) are generally less than those obtained with sine-wave stimulation, but with pulses of 8–10 msec the responses are greater.

The experiments to be described below are all concerned with studies on the effect of changing the parameters of the biphasic square-wave stimulus.

Relationship of frequency of stimulation to secretion of oxytocin. In twelve rabbits recordings of the milk-ejection responses were made following repeated stimulation using the biphasic square-wave pulses. In these experiments the pulse duration and current strength were kept constant, the variable parameter being the frequency. In seven of these experiments stimulation was applied through unipolar electrodes and in the others (five) by transversely placed bipolar electrodes.

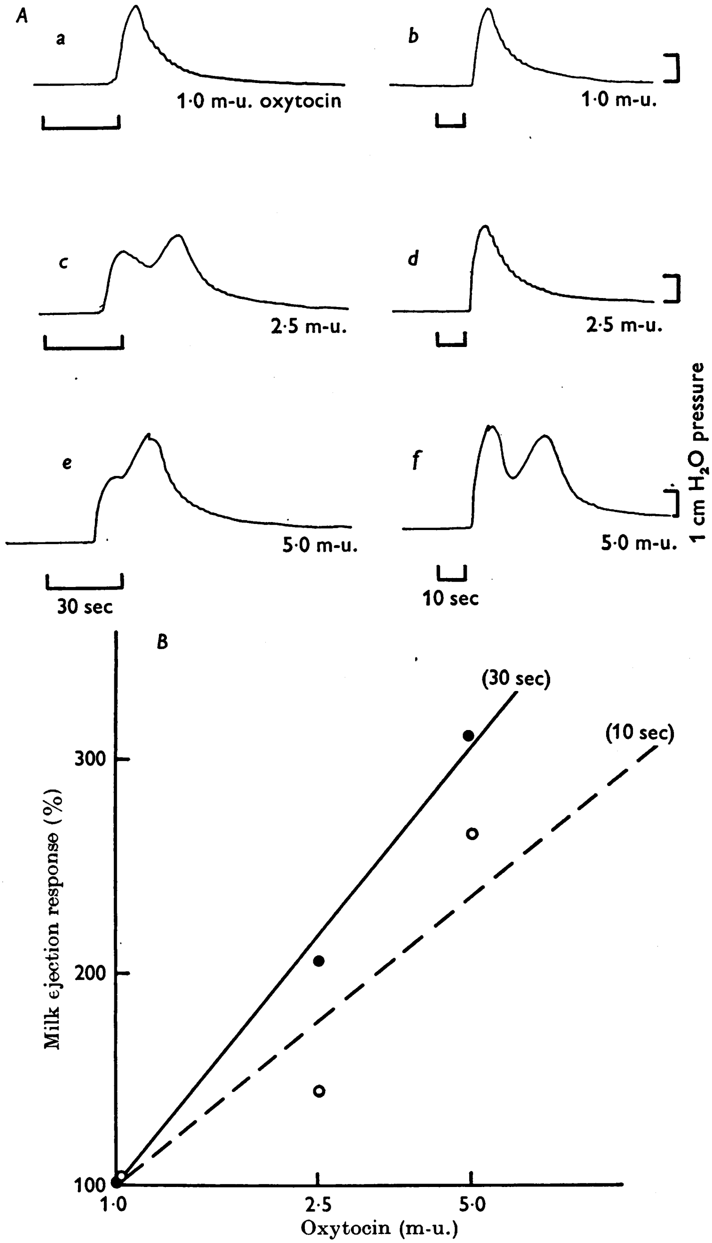

Fig. 3. (*A*) To illustrate the milk-ejection responses in a rabbit to six consecutive injections of synthetic oxytocin. The responses were obtained in the order *a*, *b*, *c*, *d*, *e*, *f*. *a*, *c* and *e* were the responses to 1, 2·5 and 5·0 m-u. oxytocin, each injection being given over a 30 sec period. *b*, *d* and *f* were the responses to similar doses of oxytocin, each injection being given over a 10 sec period. The magnitude of the responses, as measured by the area under each graph, have been plotted against the log of the dose in *B*. For comparison, the response to the lowest dose used was assigned a value of 100%.

The interesting and unexpected result of these experiments was the finding of a 'cut-out' point in the frequency range. The pulse duration was uniformly set at 6 msec. The current strength varied between 0·6 and 2·0 mA (unipolar stimulation) and between 0·15 and 0·4 mA (bipolar stimulation), but was maintained constant in any series of observations on any one animal. With stimulation in the higher frequency range

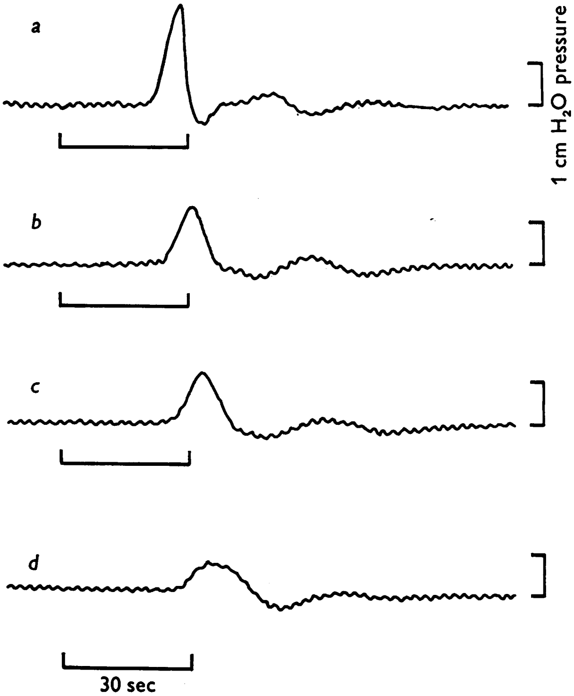

Fig. 4. To illustrate the effect of change of pulse duration on the milk-ejection response. Unipolar stimulation was applied with biphasic square-wave pulses of 50 c/s, current strength 1·0 mA, and pulse duration of (*a*) 10 msec, (*b*) 9 msec, (*c*) 8 msec, and (*d*) 6 msec. In this and subsequent experiments the period of stimulation is indicated by the vertical lines and the ripple on the base line denotes respiratory rhythm.

(40 c/s and over) a marked milk-ejection response was obtained in all animals. When the frequency of stimulation was gradually reduced a sudden diminution in the response was observed even to the point of its disappearance. This loss of response occurred over different frequency ranges in different animals (between 10 and 40 c/s), and was always reversible by repeated stimulation in the higher frequency range. An illustrative example is given in Fig. 5. The change in the response to change in frequency was similar in type whether stimulation was performed with unipolar or bipolar electrodes. It is of interest to note that repeated

observations of the 'cut-out' point in any single animal showed slight variations over long experiments (3–4 hr). This is illustrated in Fig. 5 but has been seen on several occasions.

The sudden and dramatic loss of the milk-ejection response as the frequency of the square-wave stimulus was slowly reduced raised the question as to the mechanisms involved. One possibility appeared to be that changes in frequency of stimulation and oxytocin secretion by the

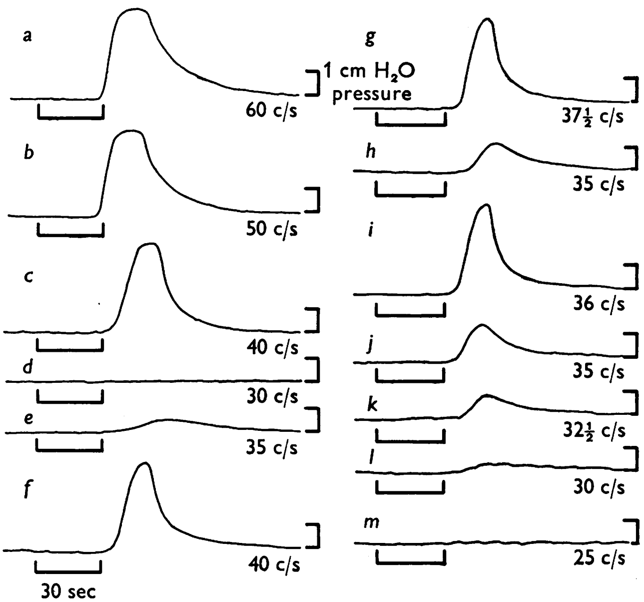

Fig. 5. To illustrate the effect of change of frequency on the milk-ejection response. Unipolar stimulation was applied with biphasic square-wave pulses of current strength 2·0 mA, pulse duration of 6 msec and frequencies of (c/s): (*a*) 60, (*b*) 50, (*c*) 40, (*d*) 30, (*e*) 35, (*f*) 40, (*g*) 37½, (*h*) 35, (*i*) 36, (*j*) 35, (*k*) 32½, (*l*) 30, (*m*) 25. Note that reducing the frequency from 40 (*c*) to 30 c/s (*d*) results in disappearance of the response but that return to 40 c/s (*f*) elicits again a marked milk ejection. Later in the experiment a slight response was obtainable at 30 but not at 25 c/s.

posterior pituitary gland were linearly related, but that the shape of the curve representing the 'cut-out' point was due in some way to the physiological response of the contractile tissue of the mammary gland to varying concentrations of oxytocin. With this in mind it became of interest to compare the curve of the responses to varying frequencies of stimulation with that of varying doses of oxytocin within the same animal. Figure 6 illustrates two such experiments, in which it may be seen that reduction of the frequency of bipolar square-wave stimulation of the supraoptico-hypophysial tract has a more marked effect in decreasing the milk-ejection response than similar proportional decreases in doses of intravenously injected oxytocin. For example, reference to Fig. 6*B* shows that stimulation at a frequency of 30 c/s results in a greater response than injection of 2·0 m-u., but that stimulation at a third of that frequency (10 c/s) does not evoke any response, whereas injection of a third of the dose of oxytocin (0·67 m-u.) still evokes a small but clear-cut effect.

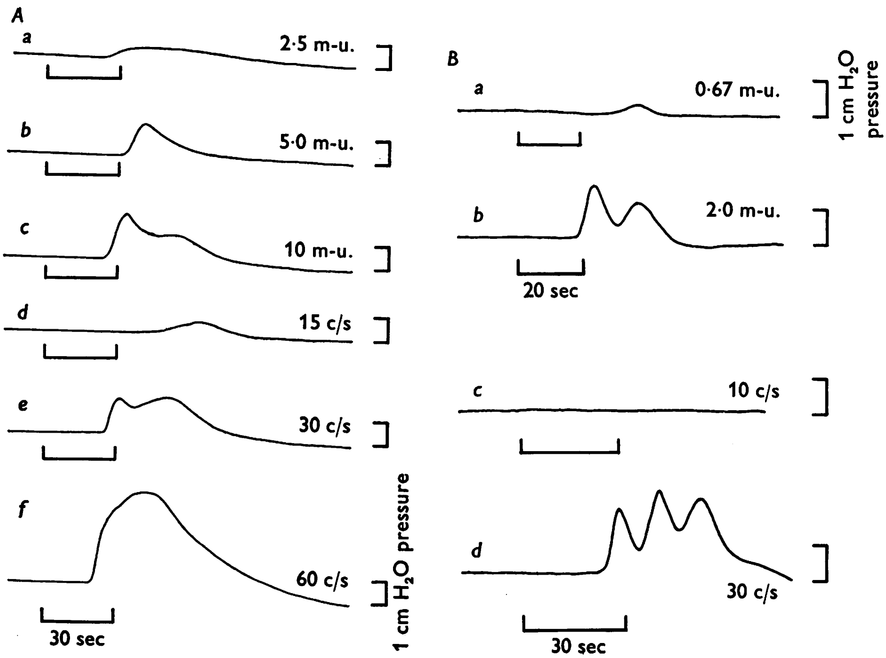

Fig. 6. To compare the effect of changing the frequency of stimulation with that of varying doses of oxytocin during the same experiment in individual animals. (*A*) Responses obtained in rabbit M 333. (*a*), (*b*) and (*c*) show the responses to I.V. injection of oxytocin (2·5, 5·0 and 10 m-u. respectively), given over a 30 sec interval (horizontal lines). (*d*), (*e*) and (*f*) show the responses to bipolar stimulation at frequencies of 15, 30 and 60 c/s respectively (at a current strength of 0·4 mA and pulse duration of 6 msec). Note that quadrupling the dose of injected oxytocin does not increase the response so markedly as does quadrupling the frequency of stimulation. (*B*) Responses obtained in rabbit M 335. (*a*) and (*b*) show the responses to I.V. injection of oxytocin (0·67 and 2·0 m-u. respectively) given over a 20 sec interval (horizontal lines). (*c*) and (*d*) show the responses to bipolar stimulation at frequencies of 10 and 30 c/s respectively (at a current strength of 0·2 mA and a pulse duration of 6 msec). This experiment is described in the text.

The effects of stimuli of graded current strengths on the secretion of oxytocin. In experiments on three rabbits a study has been made on varying the intensity of the current flow (keeping the frequency and pulse duration constant) on the milk-ejection response. In two animals unipolar stimulation was applied, and in one animal bipolar stimulation. The results showed clearly that, with constant frequency (50 c/s) and constant pulse duration (6 or 10 msec), graded milk-ejection responses are obtained for varying current strengths of up to approximately 2·0 mA. It may be said from these and other experiments that a given milk-ejection response requires a greater current strength when unipolar stimulation is applied than for bipolar stimulation.

The least strength and duration of the stimulus necessary to elicit a milk-ejection response. From the above results it seems clear that an effective stimulus to excite oxytocin secretion is given by a balanced biphasic square-wave pulse with the following parameters: peak-to-peak current strength 0·5 mA (bipolar electrodes), frequency 50 c/s, and a pulse duration of 6 msec. It was decided to investigate the least current strength and the least current duration necessary to elicit a milk-ejection response. Since repetitive biphasic stimuli were used the results obtained cannot be taken to refer to the classical strength–duration curve. However, from a practical point of view the results obtained with the use of biphasic stimuli at 50 c/s would seem to be of more value than a similar study using single undirectional shocks.

A study on five rabbits using unipolar (one) and bipolar (four) electrodes showed that with current strength of 0·5–2·4 mA, and a frequency of 50 c/s, the least duration of the square-wave pulses necessary to produce a response was between 0·5 and 1·0 msec.

A study on five rabbits, using bipolar electrodes, showed that the least current strength necessary to produce a response (with pulse duration of 6–12 msec and a frequency of 50 c/s) was approximately 0·10–0·12 mA. Stimuli of a current strength of 0·10 mA failed to elicit a response on four occasions in two rabbits, though the same animals responded to stimuli of 0·12 and 0·15 mA respectively. Two other animals responded to stimuli of 0·2 mA, though the fifth failed to respond to 0·2 mA but gave clear-cut responses to stimuli of 0·4 mA.

Other pituitary responses to square-wave stimulation. In two cases animals that had given marked milk-ejection responses to bipolar stimulation of the supraopticohypophysial tract had the electrodes fixed to stainless-steel screws inserted in the vault of the skull with dental cement and were allowed to recover from the operation. At a later date ovariectomy, vaginal transplantation, and subcutaneous implantation of a 25 mg pellet of oestradiol (Organon Laboratories, Morden, Surrey) was performed and the uterine responses to stimulation studied over a series of days. The techniques employed were similar to those of Harris (1947). It was found that square-wave pulses of similar parameters to those used for the milk-ejection experiments on these animals also evoked clear-cut oxytocic responses from the uterus. Figure 7 depicts such a uterine response.

It is of interest that square-wave pulses of similar parameters to those described above, applied to the hypothalamic regions in rats and rabbits, may induce ovulation. This has been observed many times by different workers in our laboratory (see Discussion).

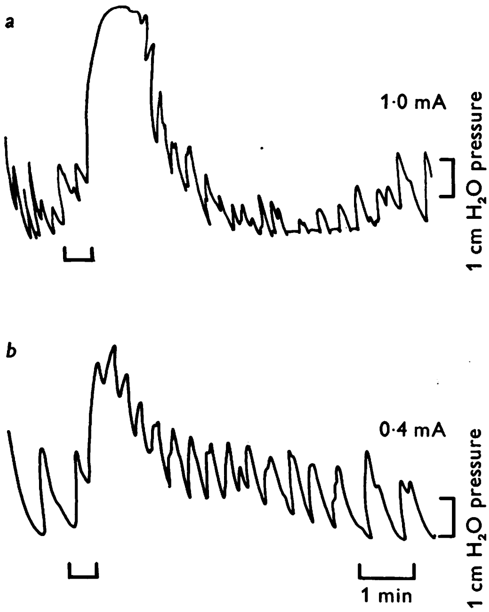

Fig. 7. To show the uterine contractions evoked by electrical stimulation of the supra-opticohypophysial tract in a rabbit in which milk-ejection responses had been obtained 16 days previously. Bipolar stimulation was applied with biphasic square-wave pulses with the following parameters: (*a*) current strength 1·0 mA, pulse duration 6 msec and frequency 50 c/s, and (*b*) current strength 0·4 mA, pulse duration 6 msec and frequency 50 c/s. In each case the period of stimulation was 30 sec (horizontal lines). Note that stimulation with parameters the same as those used in (*b*) had previously evoked a marked milk-ejection response.

DISCUSSION

It would be very difficult to assess optimum pulse parameters for electrical stimulation of the hypothalamus by experiments in which the hypothalamus itself was stimulated. This region of the brain contains such a meshwork of fine unmyelinated fibres that discrete tracts are not available for experiments of this sort. Further, it would be difficult to choose the response to measure. Any autonomic, visceral or behavioural response would have the disadvantage of being mediated by unknown pathways, probably involving a chain of short neuronal relays in the brain stem. Many of these would also be difficult to quantitate. In order to overcome these difficulties and to obtain guidance for his future experiments Hess (1932) made preliminary studies on peripheral unmyelinated fibres such as those in the cervical sympathetic chain and vagus nerve, and then applied the results to his study on hypothalamic stimulation.

Endocrine responses elicitable from the anterior pituitary gland by hypothalamic stimulation occur largely over prolonged time intervals (hours to days) and would not be suitable for repeated observations in any one animal in an acute experiment. The one clearly defined hypothalamic tract, lacking synaptic relays, and controlling a rapid and repeated response, is the supraopticohypophysial tract which regulates the secretion of vasopressin and oxytocin from the posterior pituitary gland. In the present study this tract was chosen for electrical stimulation and the milk-ejection response measured to quantitate the amount of oxytocin secreted. The infundibular stem was chosen as the site for stimulation since the tract is here separated from surrounding hypothalamic nerve fibres. The milk-ejection response was selected since it is highly specific to the action of posterior pituitary hormones, especially oxytocin. Although vasopressin has some milk-ejecting activity, and was in all probability secreted in response to stimulation, the effects described above have been referred to as oxytocin responses for the sake of brevity.

The preliminary experiments concerned with the injection of synthetic oxytocin on the milk-ejection response were conducted with three dose levels. From the few experiments made it seems that the response varies as the logarithm of the dose (see Fig. 2*B*). The possibility that the milk-ejection response is related to the logarithm of the concentration of oxytocin in the blood is of importance in assessing the response following electrical stimulation.

The aim of the present experiments has been to obtain information about the stimulus parameters that are most effective for stimulating the fibres of the supra-opticohypophysial tract, and not to localize the anatomical site from which the milk-ejection response may be elicited (see Bisset, Hilton & Poisner, 1967; Tindal, Knaggs & Turvey, 1968). Since, on occasions, it was desirable to obtain maximal responses by stimulation of all the fibres in the tract involved, the bare tip of the electrode was 1 mm in length and current strengths up to 2·4 mA have been used, from time to time.

In all experiments in the present study carefully balanced biphasic pulses, either sine wave or square wave in form, have been used since these types of wave form are easily available and reproducible in different laboratories. The risk of polarization and electrolysis, and consequent tissue damage, with the use of undirectional or monophasic pulses would seem to make the employment of such pulses inadvisable, although some recent reports refer to their use (Critchlow, 1958; Kawakami & Sawyer, 1959; Barraclough & Gorski, 1961; Hayward, Hilliard & Sawyer, 1964;

Kawakami, Seto, Terasawa & Yoshida, 1967). The comparison of the effectiveness of the sine-wave and the square-wave current pulses (for a similar frequency and peak-to-peak current strength) revealed that they were equally effective when the pulse duration of the square wave was 6 msec. At a frequency of 50 c/s this means that the mean current flow (μC) during each phase of the square-wave pulses is less than half that of the sine wave in this example. As a consequence the danger of a lesioning effect (Rowland, 1966) is less for the square-wave current. A further advantage is that the pulse duration of the square-wave current may be easily shortened or lengthened which weakens or strengthens respectively the stimulus. A possible disadvantage of the square-wave stimulus when applied to unmyelinated fibres is that any surrounding myelinated fibres have a much lower threshold for this type of stimulus than for more slowly rising and falling pulses (Hess, 1932, 1940), and therefore the spread of stimulus to surrounding myelinated tracts will be greater.

The experiments concerned with the study of changing individual pulse parameters were all made with the square-wave stimulus. As expected, the magnitude of the milk-ejection response varies, within limits, with the duration and with the strength of the pulse. Practical limits would appear to be in the range 1–10 msec pulse duration and 0·1–2·0 mA current strength (with bipolar electrodes of the design used in the present experiments). It is likely that the increasing responses over these ranges of pulse duration and current strength are due to stimulation of a greater number of nerve fibres in the tract.

The milk-ejection responses show an interesting relationship to stimuli of varying frequencies (other parameters remaining constant). With frequencies from about 100 down to 50 c/s the milk-ejection responses show a gradual diminution. At some point between 50 and 10 c/s (varying in different animals and also slightly in consecutive observations in the same animal) a sudden and abrupt diminution in the magnitude of the responses is observed over a small range of frequency. Below this frequency the response is unobtainable. In the text above this has been referred to as the 'cut-out' point. It is important to note that this is not related to 'tachyphylaxis', since repeated stimulation at the higher frequencies results in responses similar to those seen initially (see Fig. 5). In principle there seem to be three sites to which the mechanism of this 'cut-out' point could be ascribed: first, at the point of stimulation of the nerve tract in the infundibular stem; secondly, at the nerve terminals (mostly in the infundibular lobe) where the oxytocic hormone is released across the nerve membrane into the blood stream; and thirdly, at the myoepithelial contractile tissue in the mammary gland. The third possibility seems unlikely in view of the experiments in which the responses to different ratios of doses of oxytocin were compared to the responses following stimulation at different frequencies in similar ratios (Fig. 6). It is felt that the most likely site at which this 'cut-out' action occurs is at the neurosecretory nerve terminals. It is possible that a certain frequency of stimulus is necessary to give a threshold value for some reaction concerned with the neurosecretory release process.

The results of the present study indicate that a satisfactory stimulus for unmyelinated nerve fibres of the hypothalamus is given by a 50 c/s alternating sine-wave current with a peak-to-peak current strength of 0·5–1·0 mA, or a carefully balanced biphasic square wave pulse at 50 c/s with a pulse duration of 6 msec and peak-to-peak current strength of 0·5–1·0 mA.

The initial aim of these experiments was to define the parameters of sine-wave and square-wave pulses that would be effective for hypothalamic stimulation in relation to studies of anterior pituitary function. The production of ovulation, i.e. secretion of luteinizing hormone, in rabbits by hypothalamic stimulation with a sine-wave current of 50 c/s (i.e. a pulse or cycle duration of 20 msec and 0·25–1·0 mA peak-to-peak current strength) has been reported recently (Exley, Gellert, Harris & Nadler, 1968). Ovulation has been produced in rats by septal and hypothalamic stimulation with biphasic square-wave pulses by Everett (1965) and G. W. Harris & K. B. Ruf (in preparation) (with pulse parameters 30 c/s, 2 msec pulse duration and 1·0 mA peak-to-peak current strength). Similar stimuli in rabbits (Harris & Sherratt, 1969) with pulse parameters 50 c/s, 10 msec pulse duration and 1·0 mA peak-to-peak strength, also evoke ovulation. Thus similar pulse parameters seem to be effective in stimulating the unmyelinated fibres in the hypothalamus concerned with regulating the secretion of releasing factors and therefore anterior pituitary function.

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