UNIT ACTIVITY OF IDENTIFIED NEUROENDOCRINE CELLS IN CAT HYPOTHALAMUS FOLLOWING STIMULATION OF THE SEPTAL AREA AND OF THE RETICULAR FORMATION, WITH AND WITHOUT OSMOTIC STIMULI

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UNIT ACTIVITY OF IDENTIFIED NEUROENDOCRINE CELLS IN CAT HYPOTHALAMUS FOLLOWING STIMULATION OF THE SEPTAL AREA AND OF THE RETICULAR FORMATION, WITH AND WITHOUT OSMOTIC STIMULI

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Indexing Words

neuroendocrine cell;
single unit recording;
modes of spontaneous firing;
interaction of nervous and osmotic stimulation

Hiroshi KANNAN. *Unit Activity of Identified Neuroendocrine Cells in Cat Hypothalamus following Stimulation of the Septal Area and of the Reticular Formation, with and without Osmotic Stimuli.* Kobe J. Med. Sci. 20, 1-14, March 1974——The present work was designed to analyze the spontaneous discharges in the resting state of identified neuroendocrine cells in cat hypothalamus and also to examine the response and interaction of these units to electrical stimulation of the septal area (SEP) and of the mid-brain reticular formation (RF), with and without osmotic stimuli. Hemispherectomized cats anesthetized with sodium pentobarbiturate, immobilized with gallamine triethiodide were used. The supraoptic neurosecretory cells were identified antidromically by stimulation of the posterior lobe of the pituitary gland. Unit spikes were recorded extracellularly through a micropipette filled with 3 M KCl. On the basis of histograms for the firing rate of each cell, which were examined by the χ^2-test, the firing pattern was classified into three types, i.e. the Poisson distribution with mode at 1-2 spikes/sec. (51.4%); the quasi-Gaussian distribution with mode at 5 spikes/sec. (25.1%); the undefinable type (23.5%). Single or repetitive electrical stimulation of the SEP was found to induce long-lasting inhibition. Stimulation of the RF with single as well as multiple pulses yielded dual effects; i.e., facilitation and inhibition of the spontaneous activity. More than half the cells responded to stimulation of both the SEP and RF. The unit activity of the identified neuroendocrine cells induced by osmotic stimulation was affected by electrical stimulation of the SEP or RF. Interaction between neural and humoral effects on single unit had been suggested but not confirmed by experiments before I showed the evidence as described in this paper.

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INTRODUCTION

It has been proposed that antidiuretic hormone (ADH) is produced in the cell body of neuroendocrine cells of the supraoptic nucleus (SON) of the mammalian hypothalamus and is then transported to the neurohypophysis, from which it appears to be released. Much evidence has been accumulated to suggest that the release of ADH is influenced by humoral factors such as the blood osmolarity, blood volume and blood pressure.[10, 23, 27, 32] ADH release has also been observed to be affected by neural activity such as that associated with pain, emotional disturbance, exercise, stimulation of the central neural structure as well as of peripheral afferent pathways.[5, 9, 21, 26, 28, 31]

Originally, electrophysiological studies were based on units which were anatomically located in the SON and were osmosensitive.[3, 15, 18] However, the possibility cannot be completely excluded that cells other than neuroendocrine cells were also included. Recently, this problem has been overcome through the identification of neuroendocrine cells by the antidromic activation resulting from stimulation applied to the neurohypophysis.[36] Several papers describing studies on neuroendocrine cells that were identified by antidromic excitation have recently appeared.[1, 6, 7, 11, 12, 17, 19, 20, 22] However, there are relatively few reports concerning the neural control of neuroendocrine cell activity from other structures.[1, 20]

The present work was designed to analyze the spontaneous firings of neuroendocrine cells in the resting condition, and also to examine the response of these units to electrical stimulation of the septal area and of the mid-brain reticular formation, with and without osmotic stimuli.

METHODS

A total of 52 cats, weighing between 2.0 and 4.0 kg, were anesthetized with sodium pentobarbiturate, 30 mg/kg being given I.P. The cranial bone on the left side and the left hemisphere were removed to expose the pituitary gland, optic chiasma and wall of the 3rd ventricle. The three main arteries supplying the brain (Aa. cerebri ant., med. and post.) were ligated. The left femoral artery and left brachial vein were cannulated for the purpose of recording the arterial blood pressure and for giving an intravenous infusion of gallamine triethiodide, respectively. Following operation, the animals were placed in a stereotaxic instrument. Gallamine triethiodide (Flaxedil, 4 mg/kg, I.V.) was administered to them and artificial respiration was carried out to minimize body movements.

In order to identify the supraoptic neuroendocrine cells antidromically, a steel bipolar electrode was inserted into the posterior lobe of the pituitary gland. Stimulating pulses of 1.0 msec duration and between 10 and 30 V were given. Bipolar stimulating electrodes were positioned in the septal area and the mid-brain reticular formation according to the stereotaxic coordinates.[29] To stimulate these areas, 2 msec pulses were used (10-20 V). The locations of the electrode tips were examined histologically after each experiment by staining the deposited iron with 3% ferric ferrocyanide solution. For the osmotic stimulation, 0.3 ml of 1 M NaCl

was infused quickly (within about 5 sec) into the left carotid artery.

Unit spikes were recorded through a micropipette filled with 3 M KCl (5-10 MΩ). A digital computer (Nihondenshi, model JEC-7E) was employed to compute the mean firing rates, mean interspike intervals, standard deviations, coefficients of variance and autocorrelation functions, and for making histograms. Stimulus artifacts were excluded by closing a gate-circuit inserted into the recording amplifier for the period of stimulation current application.

RESULTS

1. *Identification of supraoptic neuroendocrine cells.*

Electrical stimulation of the neurohypophysis induced a unit response (Fig. 1A). The latency of the response was constant for each unit (Fig. 1B) and ranged from 9.0 to 31.0 msec in the 155 units observed. The mean latency was 15.5±3.8 (S. D.) msec. These units responded to repetitive stimuli of at least 20 Hz. With the repetitive stimuli, a notch in the rising phase of the unit spike sometimes became apparent (Fig. 1C).

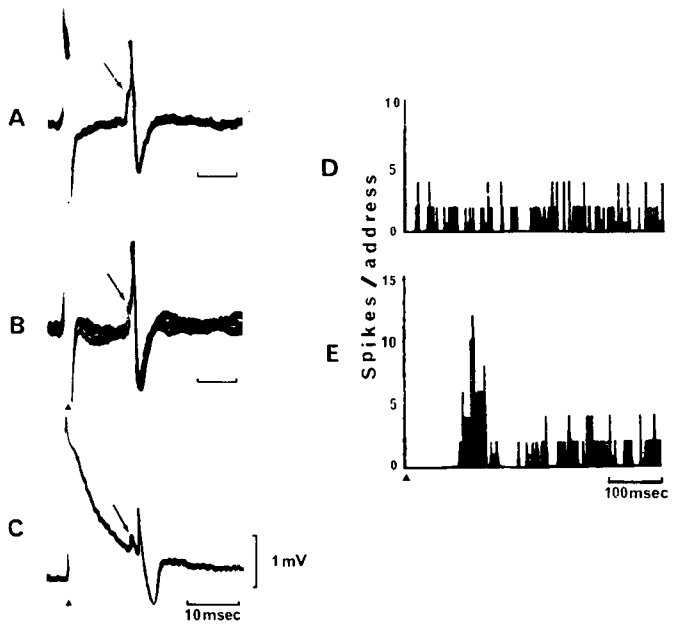

Fig. 1 A: Antidromically evoked action potential following stimulation of the neurohypophysis. The latency was 18 msec. The arrows in A~C indicate a notch in the rising phase of the antidromic action potential. B: Photograph of five superimposed tracings. C: Separation into a small spike and a large spike in the antidromic action potential following repetitive stimulation (20 Hz). D, E: Post-stimulus time histograms of unit firings in response to stimulation of the neurohypophysis. Each profile consists of 70 summated responses. D is the control. Antidromic action potentials are omitted in E.

Stimulation of the neurohypophysis sometimes inhibited spontaneous firings of supraoptic units that responded to the stimulus, during the post-stimulus period for about 100 msec (Fig. 1D, 1E). This probably represents a recurrent inhibition of supraoptic neuroendocrine cells.

Such observations have been used as criteria for designating the unit response to neurohypophysial stimulation as an antidromic response.[1,6,7,11,17,20] It is therefore concluded that the observed unit spikes were generated by neuroendocrine cells.

2. Statistical properties of spontaneous unit firings of neuroendocrine cells.

Among the 155 units examined, 123 were spontaneously firing during an observation period of more than 60 sec. The firing rates for each of the units apparently fluctuated somewhat. Statistical treatments were made for each of the units in order to test quantitatively whether or not the unit firings of the neuroendocrine cells did show any definitive firing pattern or rhythm. The mean firing rate for the 123 units was 3.60 spikes/sec and the values ranged between 0.26 and 11.57 spikes/sec (Fig. 2A). The distribution exhibited a high proportion of slow

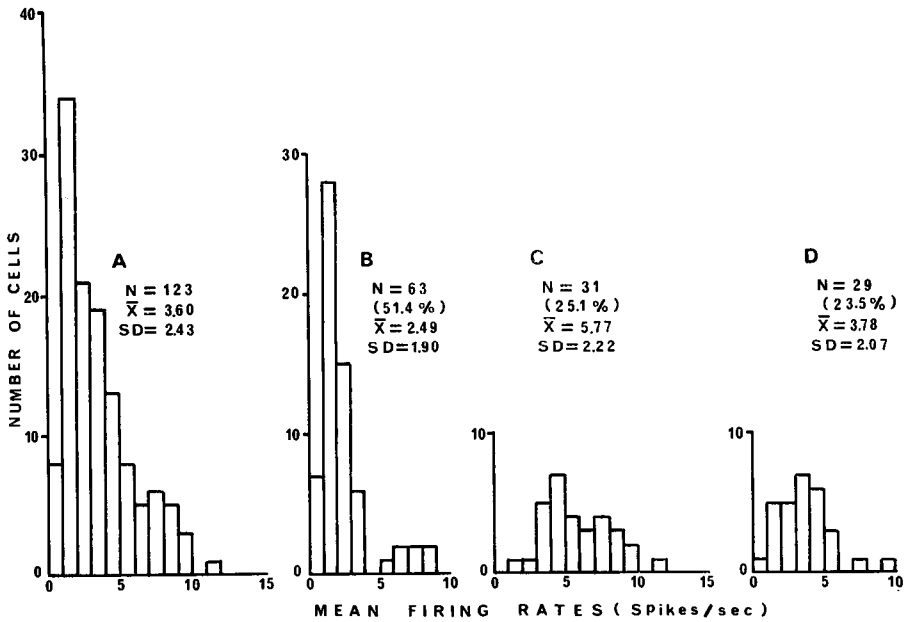

Fig. 2 A: Histogram of spontaneous firing rates of supraoptic neuroendocrine cells. N=number of samples; $\overline{X}$=mean firing rate per second observed for 60 sec; S D=standard deviation. B, C. D: Histograms of mean firing rates of supraoptic neuroendocrine cells classified by probability distributions of the firing mode. B: Poisson type. C: Quasi-Gaussian type. D: Undefined type. The numbers in parentheses indicate the proportion of units that fitted each distribution (see text).

firing units (1-2 spikes/sec.) and an exponential pattern. This result indicates that the total electrical output from the SON acts for ADH release in random fashion. Similar results were reported by others.[7, 8]

A linear relationship was observed between the mean interspike interval of an individual unit (X) and the standard deviation (Y) such that $Y = 1.02X - 0.03$ ($p<0.01$). This agrees with results previously reported for hypothalamus units.[16, 35]

Histograms of the number of spontaneous firings per second were made for each unit with a record of 60 sec. The distributions were examined by the χ^2-test ($\alpha=0.05$). The firing patterns were classified as having either a Poisson, Gaussian or skewed Gaussian distribution. Those which differed from the above mentioned distributions were classified as the undefined type. Of the 123 units examined, 63 (51.4%) were found to fit the Poisson distribution and 31 units (25.1 %) to fit the quasi-Gaussian distribution including Gaussian type (8 units). The rest of 29 units (23.5%) were classified as of undefined distribution (Fig. 2B, 2C, 2D). These results indicate that about half of the neuroendocrine cells fire with slower random frequency than some quarter cells which fire with the quasi-Gaussian type (4-6 spikes/sec).

Autocorrelation functions were calculated for each of the units with a sampling time of 1 sec and a time lag of 25 sec, but no periodicity was found at any time.

3. *Effect of osmotic stimuli on the unit firings of neuroendocrine cells.*

It has been established, following the work of Cross and Green (1959),[3] that units in the SON are sensitive to osmotic changes of the circulating blood in the carotid artery. Some differences, however, have been observed concerning the response of neuroendocrine cells to osmotic stimuli.[7, 12] Of 63 units here examined, 45 responded with an increase in firing rate after intracarotid injection of hypertonic NaCl (1.0 M, 0.3 ml). The increase in firing rate continued for 15–40 sec after the injection, followed by a gradual restoration of the pre-stimulus level within nearly 60–90 sec (Fig. 3). Three units responded with a 'biphasic' pattern;

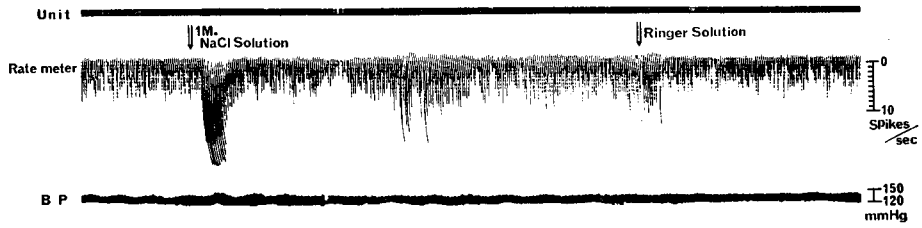

Fig. 3 Response of neuroendocrine cells to intracarotid injection of hypertonic NaCl solution (1.0 M, 0.3 ml) and isotonic Ringer's solution (0.3 ml). B P: Blood pressure.

namely, with a transient increase following a decrease in firing rate lasting for 20–40 sec. Intracarotid injection of isotonic Ringer's solution (0.3 ml) sometimes induced a small increase in unit firing but did not change the activity level of most units to any significant extent.

4. *Effect of stimulation of the septal area and RF on the unit firings of neuroendocrine cells.*

Stimulation of the septal area with a single pulse evoked spikes with a mean latency of 27 msec in 11 of 25 units examined (Fig. 4B). The latency tended to fluctuate for each unit. Such stimulation also caused a long-lasting inhibition of spontaneous unit firings. Fig. 4C represents five superimposed tracings showing

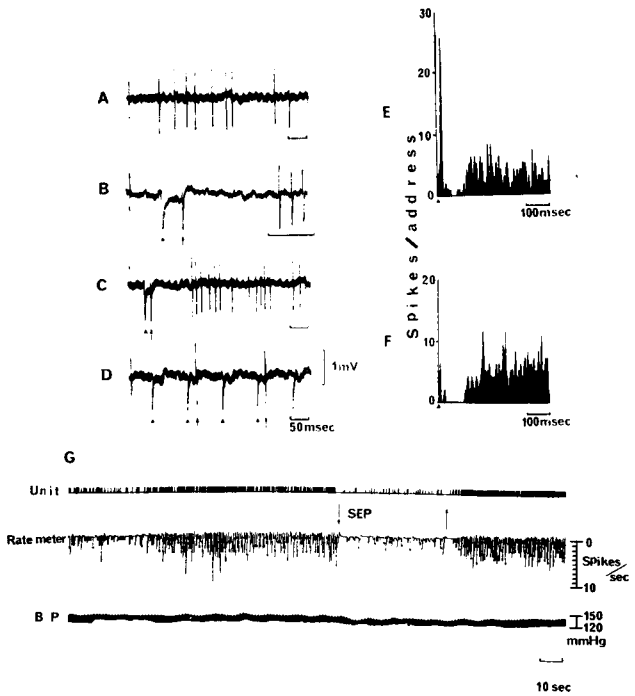

Fig. 4 Effect of septal stimulation on the unit firing of neuroendocrine cells. A shows spontaneous firings. B shows a long-lasting inhibition of spontaneous firings following stimulation (2 msec, 10V) (▲), which produced a single spike (↑) with a latency of 20 msec. C represents five superimposed tracings. D shows the response of neuroendocrine cells to repetitive stimulation (10 Hz). E and F are post-stimulus time histograms following septal stimulation. The records in E and F have been taken from other cells. Evoked spikes are seen in E but not in F. Each profile consists of 90 summated responses to the stimuli. G shows the effect on spontaneous firing rate following repetitive stimulation (10 Hz). B P: Blood pressure.

evoked spikes and a subsequent complete inhibition of spontaneous firings lasting for 100 msec. In 9 units, the inhibition also occurred without preceding evoked spikes. The inhibition of spontaneous unit firing caused by such septal stimulation was highly consistent. It was clearly apparent in the records of post-stimulus time histograms (Fig. 4E, 4F). The post-stimulus time histogram for responses to 50 or more stimuli was calculated at a sampling time of 4 msec. The mean value for the inhibitory period of spontaneous firings following septal stimulation was 110 msec (range: 65-165 msec). No significant differences in inhibitory period were observed between units with and those without evoked spikes.

Repetitive stimulation (10 Hz) of the septal area did not always induce evoked spikes but consistently blocked subsequent spontaneous firings of the neuroendocrine cells (Fig. 4D). Of 78 units examined, 55 responded with a decrease in firing rate after repetitive stimulation of the septal area (Fig. 4G). The inhibition lasted during the stimulation but there was recovery to the pre-stimulus level within a few seconds after the cessation of stimulation.

Stimulation of the RF with a single pulse evoked spikes with latencies of 10-30 msec in a few neuroendocrine cells. The stimulation also increased the spontaneous firings with variable latencies of 35-110 msec in 11 of 21 units tested,

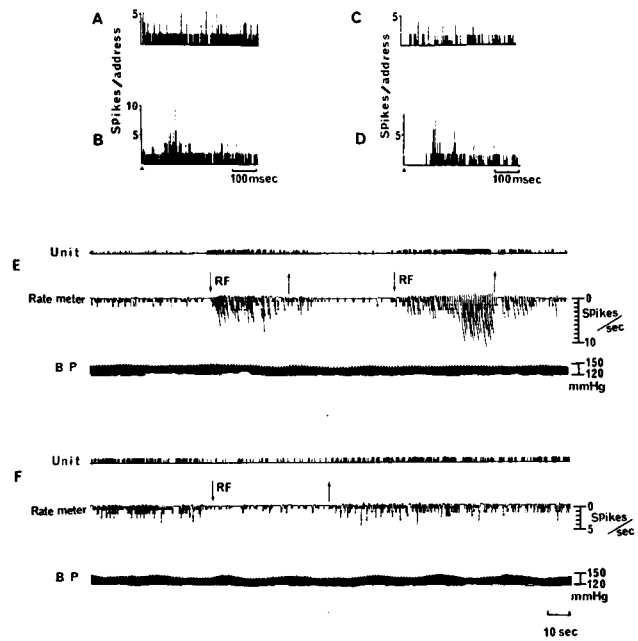

Fig. 5 Effect of RF stimulation on the unit firing of neuroendocrine cells. A, B, C and D show post-stimulus time histograms following RF stimulation (2 msec. 10 V). Each profile consists of 60 summated responses to the stimuli. A and C are controls. RF stimulation with single pulses produces facilitation (B) and inhibition (D) of spontaneous firings. E and F show the effects on unit firing following repetitive stimulation of the RF (10 Hz). B P: Blood pressure.

even when evoked spikes were not induced. The period of excitation lasted for approximately 150 msec (Fig. 5A, 5B). Six units were inhibited as regards spontaneous firing following stimulation lasting for 50-120 msec without preceding excitation (Fig. 5C, 5D).

In 31 of 59 units examined, repetitive stimulation (10 Hz) of the RF caused a significant increase in firing rate. Facilitation induced by repetitive stimuli also lasted for a few seconds after the cessation of stimulation (Fig. 5E). With higher stimulus intensities, the extent of increase was sometimes as much as about 2-3 fold. Fourteen units were inhibited by repetitive stimulation of the RF (Fig. 5F). This indicates that the septal area and RF have mixed excitatory and inhibitory effects on neuroendocrine cells.

It was also of interest to examine whether or not individual neuroendocrine cells received impulses from these areas. Repetitive stimulations of the septal area and RF were applied successively at an interval of about 60 sec. Fifteen of the 28 units examined which were inhibited by septal stimulation also responded to RF

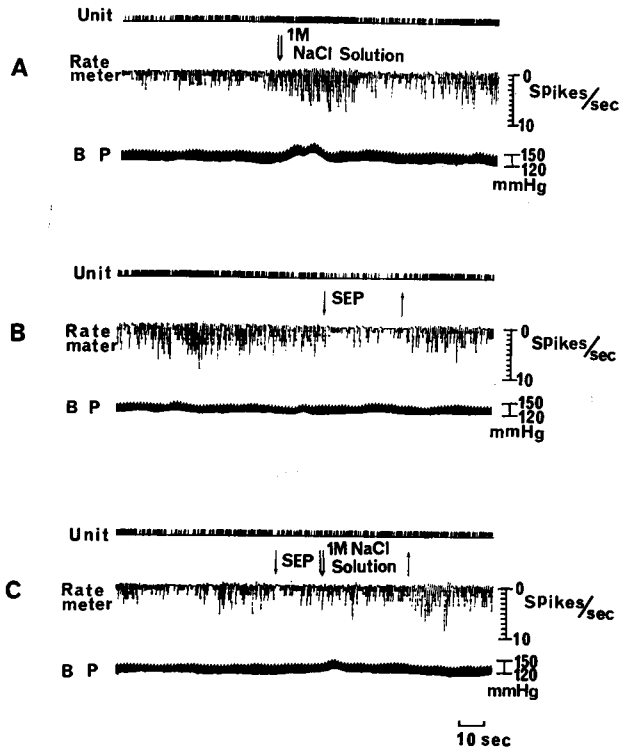

Fig. 6 A: Response of neuroendocrine cells to injection of hypertonic NaCl (1.0 M, 0.3 ml). B: Response to septal stimulation (10 Hz, 2 msec, 10 V). C: Response to a combination of the septal and osmotic stimuli. (Note the suppression of the response to the osmotic stimulus following septal stimulation.) B P: Blood pressure.

stimulation. Eight units were excited and 7 units were inhibited. The remaining 13 units were unresponsive to stimulation of the RF. This suggests that various signals from the septal area and RF converge on individual neuroendocrine cells.

5 *Effect of stimulation of the septal area and RF on the response of neuroendocrine cells to osmotic stimuli.*

Intracarotid injection of hypertonic NaCl solution (1.0 M, 0.3 ml), repetitive stimulation (10 Hz) of the septal area or RF, and a simultaneous combination of both stimulations were performed successively with an interval of about 60 sec, in order to elucidate the interaction of osmotic and neural stimuli on neuroendocrine cells. Four of 7 units examined responded with a suppression of facilitation induced by osmotic stimuli after stimulation of the septal area (Fig. 6). The remaining 3 units did not change their responses to the osmotic stimuli following stimulation of the septal area.

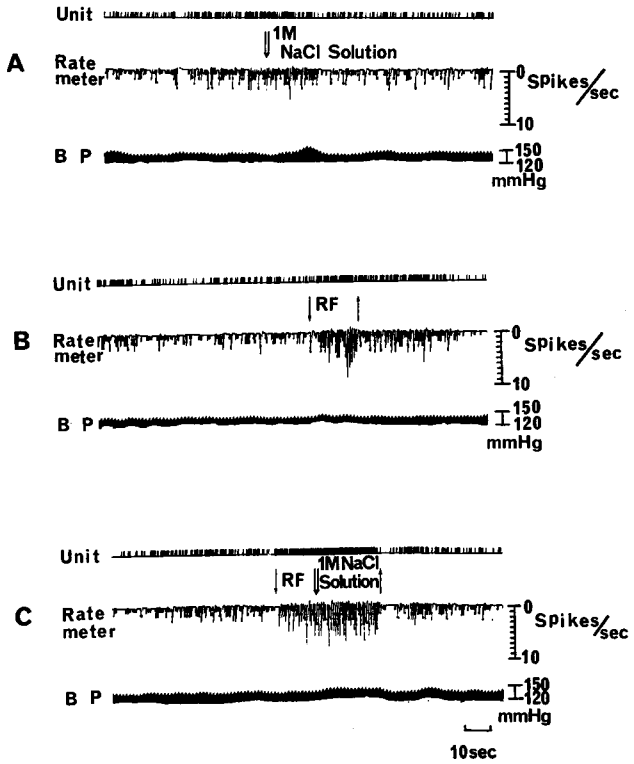

Fig. 7 A: Response of neuroendocrine cells to injection of hypertonic NaCl (1.0 M, 0.3 ml). B: Response to RF stimulation (10 Hz, 2 msec, 10 V). C: Response to a combination of RF stimulation and osmotic stimulation. (Note the approximate summation of responses during this simultaneous stimulation.) B P: Blood pressure.

When the osmotic stimulation was performed following repetitive stimulation of the RF, 4 of 7 units examined, which were excited by the RF stimulation, responded in a manner which approximated the sum of their individual responses to the different stimuli (Fig. 7). The remaining units did not change their responses to the osmotic stimuli following RF stimulation. This indicates that the neural activity level of neuroendocrine cells induced by a change in blood osmolarity is modified by various signals from the septal area and RF.

DISCUSSION

1. *Properties of spontaneous unit firings of neuroendocrine cells.*

The present results demonstrate that about 80% of neuroendocrine cells are continuously active and that their spontaneous firing rate is rather slow. This proportion of 'silent' units, and the mean firing rate observed, are similar to those reported by other authors.[1, 7, 8]

The finding that the neuroendocrine cells could be distinguished statistically into two groups, i. e., a slow and a fast firing unit or Poisson and Gaussian type, was also supported by other observations.[7] A cause of subgroup formation may be ascribed to variety of pattern of the input activity. This idea is also supported by the facts that a pattern of the cell classification based on the firing rate changes in another form by making a hypothalamic island.[8] It has also been reported that three types of neuroendocrine cells are randomly distributed in the SON with each type receiving specific input, and their cell groups may be related to the cellular secretion of specific neurohypophysial hormones and neurophysins.[11] These three types show 'continuously active', 'phasic' or 'burster' and 'silent' firing patterns.[6, 7, 11] Neuroendocrine cells with a 'phasic' firing pattern, or periodicity, however, were not detected in the present experiments. This is probably attributable to differences in experimental conditions, such as the anesthetic agents used, animal species and operative procedures. The influence of anesthetic agents on the unit firing of neuroendocrine cells, as well as that of other hypothalamic cells, has been demonstrated by several authors.[2, 14] In addition, it must always be remembered that the sampling period and number of samples examined may have an important bearing on the analysis of the firing pattern and periodicity.[24] The author, however, prefers to consider that neuroendocrine cells as a cell group fire randomly by means of the convergence of impulses from different areas. The possibility also exists that a random state of neural activity is a suitable 'waiting' state for the control mechanism of ADH release. Some small influence, be it neural or osmotic, would then be sufficient to call many neuroendocrine cells promptly into simultaneous action, providing a finely tuned control for maintaining a stable level of blood ADH.

2. *Neural control of neuroendocrine cells from the septal area and RF.*

It has long been known that ADH release is closely related to emotional stresses such as pain.[5] There is also anatomical evidence to show that the limbic

system and mid-brain reticular formation are intimately concerned in the emotional state projected to the SON as well as the paraventricular nucleus.[25,34] Moreover, it has been demonstrated that ADH release can be induced by electrical stimulation of these areas, such as of the amygdala, hippocampus and mid-brain central gray region.[13, 28] The present results reveal that neuroendocrine cells respond with a brief excitation period followed by a long-lasting inhibition of septal stimulation. The inhibition can occur in the absence of preceding excitation and is generally clear following repetitive stimulation. Stimulation of the RF produces dual effects; namely, excitation and inhibition of the neural activity of neuroendocrine cells, with a long latency, suggesting that these responses occur by polysynaptic pathways. The finding that the septal areas and RF have a dual effect on neuroendocrine cells agrees with other reports.[20] The results reported here also demonstrate that various signals from the septal area and RF converge on individual neuroendocrine cells. Since both these areas are considered comprehensive areas, anatomically connected with other central neural structures, the physiological role of the septal area and RF in the regulation of neuroendocrine cells needs further clarification. It is, however, concluded that the septal area and RF are at least involved in modulatory mechanisms buffering the neural activity level of neuroendocrine cells, be it inhibitory or excitatory.

3. *Interaction of neural and humoral stimulation of neuroendocrine cells.*

There is little detailed information available on the degree to which humoral and neural factors are quantitatively responsible for control of the neural activity of neuroendocrine cells. It has been observed that the neural activity of certain cells in the hypothalamus, including the supraoptic osmosensitive cells, is augmented after their isolation from the surrounding structures, indicating that constant inhibitory influences from elsewhere are normally exerted over them.[4, 30] The present results show that the response of neuroendocrine cells to osmotic stimuli is affected by stimulation of the septal area and RF, indicating the possibility of a situation whereby neural control, in preference to humoral control, could play a more important role in the regulation of the level of neural activity of neuroendocrine cells. Moreover, the finding that some neuroendocrine cells have their responses to osmotic stimuli inhibited following electrical stimulation tends to support the idea that neuroendocrine cells might be controlled antagonistically by inhibitory neural and facilitatory humoral factors as Koizumi and Yamashita mentioned.[20] It has been observed, however, in studies on cats and rats with a deafferented hypothalamo-hypophysial island, that water balance can be effectively managed under various conditions involving different physiological stimuli.[33] It thus seems more reasonable to consider that the level of neural activity of neuroendocrine cells maintained by a reaction to the humoral environment is rapidly modified by many neural impingements via various neural pathways, be it through direct inhibition or through the removal of excitation. In this way, stable and prompt control of ADH release is thought to be maintained.

SUMMARY

1. Extracellular single unit recordings were made from antidromically activated supraoptic cells of hemispherectomized cats. Units were identified to be antidromic if they met the criteria for stable latency and response to repetitive stimulus of the neurohypophysis.

2. The properties of spontaneous unit firings of the neuroendocrine cells were examined. The mean firing rate was 3.6 spikes/sec. The relationship between the mean interspike interval and standard deviation was linear. Histograms for the number of spontaneous unit firings per second fitted with a Poisson distribution in 63 of 123 units examined and with a quasi-Gaussian distribution in 31 units. The fast and slow firing groups could be separated. No periodicity in the unit firings was observed.

3. Intracarotid injection of hypertonic NaCl solution caused an increase in unit firing of the neuroendocrine cells.

4. Single pulse stimulation of the septal area was found to induce evoked spikes, followed by a long-lasting inhibition of spontaneous unit firing. Repetitive stimulation caused a decrease in spontaneous unit firings.

5. Stimulation of the mid-brain reticular formation with a single pulse, as well as with multiple pulses, yielded dual effects; namely, excitation and inhibition of the unit activity of the neuroendocrine cells.

6. More than half the units responded to stimulation of both the septal area and RF.

7. The unit activity of neuroendocrine cells induced by osmotic stimulation was affected by electrical stimulation of the septal area or RF.

8. The above results are discussed in relation to an integrative mechanism for neuroendocrine cells in response to neural and osmotic stimuli.

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