LITERATURE CITED

1. O. S. Andrianov, Principles of Organization of Integrative Activity of the Brain [in Russian], Moscow (1976).
2. A. S. Batuev, Cortical Mechanisms of Integrative Activity of the Brain [in Russian], Leningrad (1978).
3. A. G. Polyakova, Functional Organization of the Association Cortex [in Russian], Moscow (1977).
4. A. G. Polyakova, Zh. Vyssh. Nerv. Deyat., No. 4, 827 (1975).
5. A. G. Polyakova, Neirofiziologiya, 10, 573 (1978).
6. N. S. Popova and L. M. Kashalova, Zh. Vyssh. Nerv. Deyat., No. 4, 718 (1978).
7. H. Asanuma, Physiol. Rev., 55, 143 (1975).
8. T. Desiruju, Brain Res., 109, 473 (1976).
9. M. Imbert, K. Bignall, and P. Buser, J. Neurophysiol., 29, 382 (1966).
10. J. Joseph, Rev. Electroenceph., 6, 221 (1976).
11. J. D. Newman, J. Med. Primatol., 4, 391 (1975).

QUALITATIVELY SIMILAR EFFECTS OF MICROIONTOPHORETIC APPLICATION OF CORTICOTROPHIN AND HYDROCORTISONE TO HIPPOCAMPAL AND HYPOTHALAMIC NEURONS IN RABBITS

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KEY WORDS: Microiontophoresis; neurons of the hypothalamus and hippocampal septum; hydrocortisone; ACTH.

The work of H. Selye [11] laid the foundations of extensive research into the role of pituitary-adrenal hormones in adaptive mechanisms aimed at increasing the resistance of the body to stresses of varied origin. However, the problem of the reverse effect of adrenocortical and pituitary hormones on the CNS in stress has been incompletely studied. In particular, we do not understand the reasons for the difference and similarity between the effects of corticoids and corticotrophin (ACTH) on nerve cells in different parts of the brain. All that is known is that, with an increase in the concentration of corticoids and ACTH in the hypothalamic region in experimental animals, secretion of anterior pituitary hormones is inhibited and this leads to corresponding weakening of the secretory activity of the adrenal cortex [5-7, 9].

The object of this investigation was to study differences and similarities in the character of the effect of ACTH and hydrocortisone on spontaneous unit activity in the hypothalamus and in limbic structures of the brain such as the septum and hippocampus which play an important role in the regulation of secretion of pituitary-adrenal hormones. The method of microiontophoretic application of hormones to the surface of the nerve cells to be studied was used in the investigation, so that the presence of true sensitivity of a particular nerve cell to the hormone applied could be more definitely decided.

EXPERIMENTAL METHOD

Responses of 79 neurons in the hypothalamus and in the region of the septum and dorsal hippocampus to application of ACTH and hydrocortisone were studied in acute experiments on 20 adult male rabbits fixed in a stereotaxic apparatus. Microiontophoretic applications of the substances [4] were made by means of multiple-barreled glass microelectrodes [1]. Bovine ACTH in a concentration of 0.5 mM and hydrocortisone hemisuc-

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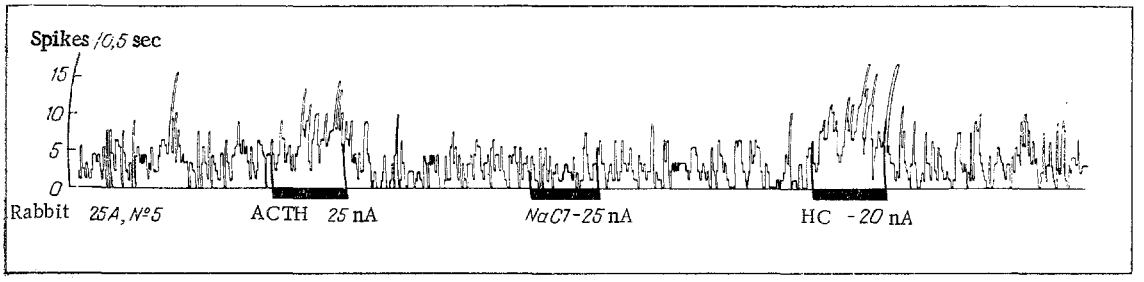

Fig. 1. Increase in discharge frequency of hypothalamic neuron in area CA_2 in experiment on rabbit in response to microiontophoretic application of ACTH and hydrocortisone (HC). Counting period of histogram 0.5 sec. Control injection of physiological saline (NaCl) does not change cell discharge frequency. Rabbit No. 25A, No. 5 denotes laboratory serial numbers of animal and neuron.

TABLE 1. Response of Neurons to Two Hormones

Response to ACTH	Response to hydrocortisone											
	HYM HDM			AHL			NSL, NSM			hyppo-campus		
	slowing	0	quickening	slowing	0	quickening	slowing	0	quickening	slowing	0	quickening
Slowing	4	3	—	1	1	1	—	1	1	4	3	—
No response	1	9	—	1	10	—	2	5	3	—	7	2
Quickening	1	3	1	—	3	—	1	2	2	—	2	5
n	22			17			17			23		
K	+0,44			+0,47			+0,05			+0,62		
P	<0,95			<0,95			<0,95			0,99		

Legend. 0) No response. Numbers underlined once denote responses in same direction, numbers underlined twice denote responses of antagonistic character.

cinate (Roussel: 0.1 mM) were applied extracellularly by anionic currents (10-80 nA) for 20-40 sec. The momentary mean discharge frequency of the nerve cells before and during application was analyzed by means of the NTA-1024 pulse analyzer (Hungary). Slowing or quickening of discharges of a nerve cell by more than 30% during application of the hormone compared with the preceding spontaneous frequency was regarded as an inhibitory or excitatory response of the neuron. The location of the neurons was determined histologically by pontamine labeling in serial sections by a freezing technique. The significance of coefficients of correlation was calculated by the chi-square test [2].

EXPERIMENTAL RESULTS

Histological analysis of the location of the electrode tips revealed that all nerve cells studied were in the brain. For instance, 22 of the 79 neurons were in the medial hypothalamus – in the ventromedial (HVM) and dorsomedial (HDM) nuclei of the hypothalamus (according to Fifková and Marsala's atlas [3]). There were 17 cells in the region of the lateral hypothalamic area (AHL), and also 17 cells in the medial (NSM) and lateral (NSL) septal nuclei, and 23 neurons in the dorsal hippocampus (areas CA_2 and CA_3). During microiontophoretic application of hormones, the discharge frequency of some of the cells changed after a latent period of 0.5 to 2-5 sec. After stopping the current, these phenomena usually did not cease at once but continued a little longer (up to 10 sec). A continuous frequency histogram of discharges of one hippocampal neuron of a rabbit is illustrated in Fig. 1. The response of quickening of the discharges in this neuron arose after application of both ACTH and hydrocortisone. Control injections of physiological saline using a current

TABLE 2. Action of Hormones on One Brain Neuron

Response to ACTH	Response to hydrocortisone		n_2
	slowing	quickening	
slowing	9	2	11
quickening	2	8	10
n_1	11	10	21
	$r=+0,62$	$P=0,99$	

of the same strength, in this case as in most others, caused no change in discharge frequency, evidence of the absence of any direct effects of the current in the genesis of responses to application of the hormones. Experimental data on the character of response of neurons in different parts of the brain to successive applications of two hormones to the same neurons are given in Table 1. They show that some cells either did not respond or, on the contrary, they responded at once to both hormones, whereas other cells responded to only one of the physiologically active substances.

Calculation of coefficients of correlation (K) of mutual coordination of responses to each hormone in different brain regions showed that such correlation in the septum was virtually absent (K = 0.05). Considerable positive correlation, approaching a significant level, was observed in both the medial and the lateral hypothalamus. Closest correlation between sensitivity of the neurons to the two hormones was found in the dorsal hippocampus (K = +0.62, P = 0.99); 9 of 23 neurons responded in the same direction to the hormones; 7 were absolutely insensitive. The cells never gave antagonistic responses. The magnitude of the coefficients in Table 1 is largely dependent on the presence of a reactive neurons, the functional role of which is unknown. By choosing from the total number of cells tested only those showing sensitivity to both hormones, and presenting them in Table 2, some idea can be obtained on the similarity and difference between the action of each hormone on the same brain neuron. The results showed that most cells (17, the numbers underlined once in Table 1) responded in the same direction and only in four cases were the responses antagonistic in character. Two such cells were found in the region of the septum, one each in the lateral and medial hypothalamus (numbers underlined twice in Table 1). Nevertheless, general predominance of concordant responses revealed positive correlation (r = 0.62, P = 0.99, Table 2) between the character of responses of nerve cells in the hypothalamic and limbic structures of the brain to application of ACTH and hydrocortisone. From this point of view our findings differ from the results and conclusions given by Steiner [12, 13], who found in experiments on rats anesthetized with urethane that most cells responded to microiontophoretic applications of ACTH by quickening, and to applications of the steroid preparation dexamethasone phosphate (DMP) by slowing of the discharge frequency. On the basis of these observations, Steiner concluded that the action of ACTH and of steroids is antagonistic in character at the level of hypothalamic neuronal mechanisms. The possibility cannot be ruled out that the DMP preparation used by Steiner is not absolutely steroid-like in its action when applied directly to the surface membrane of central neurons. Indirect confirmation of this suggestion may be provided by an investigation by Koranyi et al. [8], who studied the action of systemic injections of ACTH and hydrocortisone on multicellular activity of the medial preoptic area of the hypothalamus. They found that the discharge frequency of neurons in this region of the brain fell similarly as a result of the action of both hormones. It is also known [10] that injections of DMP did not change unit activity in the basal hypothalamus, whereas injections of ACTH caused an increase in their discharge frequency.

Nerve cells of different brain structures thus differ from each other in the adequacy of their response to ACTH and hydrocortisone. Most neurons of the dorsal hippocampus have similar sensitivity to both hormones. In the medial and lateral hypothalamus there is a corresponding tendency for neurons to respond in the same direction to these two hormones. Only in septal nuclei was no correlation found between the direction of the responses to the two hormones. Cells with concordant and contradictory responses were found with equal frequency in this region.

LITERATURE CITED

1. V. P. Belyi and V. V. Sherstnev, Zh. Vyssh. Nerv. Deyat., No. 6, 1321 (1973).
2. N. A. Plokhinskii, Biometrics [in Russian], Moscow (1970).
3. E. Fifková and J. Marsala, in: Electrophysiological Methods of Investigation [Russian translation], edited by J. Bures, M. Petran, and I. Zachar, Moscow (1962), p. 386.
4. E. Endroczi, K. Lissak, and M. Tekeres, Acta Physiol. Acad. Sci. Hung., 18, 291 (1961).

5. B. Halasz and J. Szentagothai, Acta Morphol. Acad. Sci. Hung., 9, 251 (1960).
6. J. W. Kendall, D. M. Cook, and L. Tang, in: Anatomical Neuroendocrinology, edited by W. E. Stumpf and L. D. Grant, Basel (1975), p. 276.
7. L. Koranyi, C. Beyer, and C. Cuzman-Flores, Physiol. Behav., 7, 331 (1971).
8. M. Motta, G. Mangili, and L. Martini, Endocrinology, 77, 392 (1965).
9. C. H. Sawyer, M. Kawakami, B. Meyerson, et al., Brain Res., 10, 213 (1968).
10. H. Selye, The Physiology and Pathology of Exposure to Stress, Montreal (1950).
11. F. A. Steiner, Prog. Brain Res., 32, 102 (1970).
12. F. A. Steiner, in: Anatomical Neuroendocrinology, edited by W. E. Stumpf and L. D. Grant, Basel (1975), p. 270.

COUPLING OF MEMBRANE ELECTRICAL PROCESSES AND CONTRACTILE ACTIVITY OF SMOOTH MUSCLE CELLS OF THE ANOCOCCYGEUS

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KEY WORDS: Smooth muscle cells; phasic and tonic contraction; calcium channels; blockers of the calcium current; depolarization.

A close connection is known to exist between the degree of polarization of the membrane and contraction of smooth-muscle cells (SMC) [3, 5, 6, 8]. The inflow of external calcium is considered to be the chief component in the coupling of excitation with contraction [2, 4, 6, 9]. However, according to some workers, an effect of intracellular calcium, which is connected with slow (tonic) contraction [7], is another possibility.

The object of this investigation was to study how activation of SMC of the anococcygeus muscle depends on membrane potential (MP), by the use of various agents blocking the calcium current.

EXPERIMENTAL METHOD

Experiments were carried out on strips of the rabbit anococcygeus muscle 200 μ in diameter and 1.5 cm long. Electrical activity of SMC of the anococcygeus was recorded by the double sucrose gap method [1], with simultaneous recording of contractile activity of SMC. Changes in the degree of membrane polarization were produced both by changing the external K^+ concentration and by the action of a polarizing current. The original Ringer-Locke solution (35°C) was of the following composition (in mM): NaCl 154.0, KCl 5.1, $CaCl_2$ 2.2, $NaHCO_3$ 1.8, glucose 5.6. Changes in the external K^+ concentration were produced by removal of KCl or addition of the dry salt to the Ringer-Locke solution.

EXPERIMENTAL RESULTS

In most cases the anococcygeus muscle cells possess spontaneous electrical and contractile activity. Accordingly, experiments were carried out on muscle strips in which spontaneous activity was weak or completely absent, for such activity would complicate the course of the investigation.

A depolarizing current of 10 μA, 100 msec in duration, evoked action potentials (AP), but a depolarizing current (1 μA) led to the appearance of an anelectrotonic potential (AET) in the muscle cells (Figs. 1a, b: K_0, A_0). The action of a hyperpolarizing current on SMC of anococcygeus normally does not cause relaxation of the muscle, regardless of the strength of stimulation used (Fig. 1a, b: A_0). The AP of anococcygeus SMC con-

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