the delayed inactivation of GABA produced by it may be the reason why depakine delays the awakening of hibernating animals when administered in doses much smaller than those in which it causes GABA accumulation. It has been shown that the highest concentration of endogenous GHBA, 13–15 times higher than in brain tissue, has been shown to be a feature of the brown fat [9]. Considering the important role ascribed to brown fat in the maintenance of temperature regulation [5], it can be tentatively suggested that this fact is in agreement with the writers' hypothesis of the possible role of GHBA as one of the biologically active substances responsible for the unique pattern of metabolic regulation in hibernating animals. However, it has already been stated that the inhibitory effect of GHBA and depakine on behavioral awakening was more marked than their action on temperature regulation. This suggests that GHBA not only induces a metabolic effect, but also participates in the central mechanisms maintaining this unique adaptive state, namely natural hibernation.

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ELECTROPHYSIOLOGICAL AND CATECHOLAMINE MECHANISMS OF NEGATIVE FEEDBACK IN HYPOTHALAMIC REGULATION OF THE MALE GONADS

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A very important aspect of the problem of control over male gonad function is the relationship between the hypothalamo–hypophyseo–gonadal system (HHGS) and the sympathicoadrenal system (SAS). It has been shown that catecholamines play a determining role in the mechanism of secretion of gonadal releasing hormones [4, 7, 8, 11, 12], and in particular, that excitation of dopaminergic structures of the mediobasal hypothalamus is accompanied by activation of testicular function [8, 9]. These facts indicate that dopamine can be regarded as the leading mediator in the mechanism of direct positive communication at the level of the stage of HHGS control. However, the question of its participation in the realization of negative feedback, and also the role of other hormones and mediators of the catecholamine group requires further research. Hyperandrogenization,

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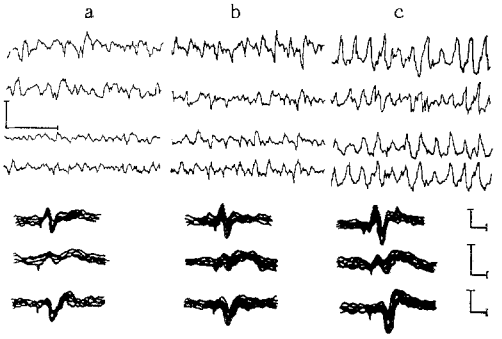

Fig. 1

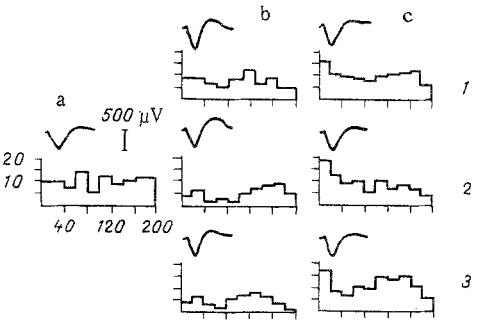

Fig. 2

Fig. 1. EEG and EP in initial state (a) and 30 min (b) and 1 h (c) after injection of testosterone. From top to bottom: EEG of left sensomotor cortex, right sensomotor cortex, medial thalamus, AR of hypothalamus, EP of sensomotor cortex, medial thalamus, and AR of hypothalamus. Calibration: for EEG 1 sec, 100 μV; for EP 20 msec, 200 μV.

Fig. 2. Poststimulus histograms of frequency of EUA and averaged FER from hypothalamic AR. a) Initial state, b) after injection of testosterone, c) after injection of peach oil. 1) After 30 min, 2) after 1 h, 3) after 2 h. Abscissa, time (in msec); ordinate, number of spikes.

due to an excess of endogenous or exogenous male sex hormones, is known to lead not only to a disturbance of direct positive communication with HHGS, but also to considerable disintegration in the activity of SAS, possibly resulting in cardiovascular dysfunction and endocrine psychoses. Meanwhile the primary disturbances of SAS lead to significant shifts in HHGS. Existing electrophysiological and biochemical data on a combined disturbance of the activity of HHGS and SAS during hyperandrogenization are extremely limited and largely contradictory, due to the heterogeneity of the experimental and clinical material, the use of unstandardized techniques and of a wide range of doses and methods of administration of androgens. The exceptions are studies of spontaneous spike generation by neurons of testosterone-reacting regions of the hypothalamus during artificial hyperandrogenization [1, 2], but they are concerned with delayed reactions and not with changes in excitability of the neurons studied or their mediator nature.

This paper describes an attempt at combined study of the hypothalamic component of HHGS, namely the arcuate region (AR), and SAS in the early stages of hyperandrogenization in order to shed light on the immediate electrophysiological and also the mediator mechanisms of negative feedback.

EXPERIMENTAL METHOD

Hyperandrogenization was produced in mature male albino rats weighing 180–200 g by intramuscular injection of an oily solution of testosterone propionate in a dose of 30 mg/kg body weight. Adrenalin (A), noradrenalin (NA), dopamine (DA) and dopa were determined in tissues of the cerebral cortex, hypothalamus, adrenals, liver, and heart and in the blood by the method of Von Euler and Lishajko in Matlina's modification. Evoked potentials (EPs) in response to electrodermal stimulation of the forelimb (square pulses 1 msec in duration) in the hypothalamus, sensomotor cortex, and medial thalamus were recorded in animals anesthetized with pentobarbital by means of stereotaxically implanted nichrome electrodes [10] on the UÉFPT–5 apparatus by superposition on photographic film, and the EEG was recorded on the ÉÉG P4–02 electroencephalograph. The focal evoked response (FER) and evoked unit activity (EUA) in AR to electrodermal stimulation of the zone of the vibrissae (square pulses 0.2 msec in duration) in animals immobilized with tubocurarine were recorded by means of glass microelectrodes (diameter of tip 4–5 μ) filled with 3 M NaCl solution (resistance not more than 1 MΩ) from an S1–18 oscilloscope by means of the FOR–2 camera. The transmission band of the amplifier was 0.5–2000 Hz, so that EUA and FER could be recorded in the same channel. The location of the microelectrode track in each experiment was confirmed histologically in frontal serial brain sections. Amplitude–time parameters of EP and FER and biochemical parameters were subjected to statistical analysis by Student's test [5]. The biochemical investigations were carried out in the initial state and 1 h after injection of testosterone propionate, the electrophysiological investigations in the initial state and 30 min and 1, 2, and 3 h after injection. Intramuscular injection of peach oil in the same volume as the testosterone (0.05 ml) was used as the control. The biochemical tests were carried out on 30 animals and the electrophysiological on 40.

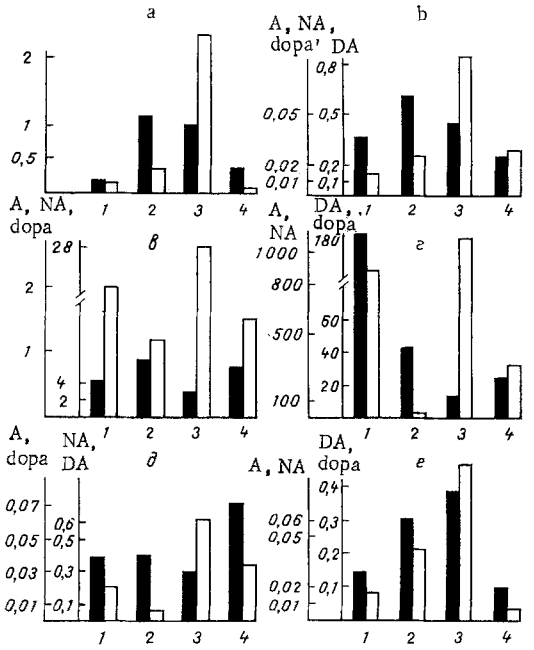

Fig. 3. Catecholamine levels in tissues and blood 1 h after injection of testosterone (unshaded columns) compared with initial state (shaded columns). Ordinate, hormone concentration (in μg/g; a) hypothalamus, b) cerebral cortex, c) blood, d) adrenal, e) myocardium, f) liver. 1) A, 2) NA, 3) DA, 4) dopa.

EXPERIMENTAL RESULTS

Integral electrical activity of the hypothalamus and cerebral cortex and, to a lesser degree, of the medial thalamus 1–2 h after injection of testosterone was characterized by an increase in the relative contribution of ν-waves (Fig. 1). The EPs during these periods showed a statistically significant increase in amplitudes of the first positive and negative components in the hypothalamus, and a less marked increase in the cerebral cortex and thalamus (Fig. 1). In control experiments changes in these parameters were less significant and, more important, they were of shorter duration: Their duration did not exceed 1 h, whereas after injection of testosterone they were recorded for 3 h.

The results of analysis of EUA were as follows. In the initial state a small decrease in the number of spike discharges was found in the initial segment, but an increase was found on the ascending region and peak of the first negative component of FER (Fig. 2a). Conversely, 1–2 h after injection of testosterone, a distinct decrease in the frequency of spike discharges was observed in the period of negativity of FER. The frequency of EUA increased somewhat but only on the descending part of the negative component (80–100 msec after the stimulus) and later (Fig. 2). This character of EUA continued for 2 h after injection of the compound, and most important, it was recorded against the background of shortening of the first positive component and a statistically significant increase in the amplitude of the negative component, i.e., changes taking place in EP also. Injection of peach oil in the control animals was unaccompanied by any visible changes in FER or depression of EUA. Moreover, an increase in frequency of spike discharges was clearly observed at the stage of formation of positivity of the FER (Fig. 2c).

Analysis of individual neuronal evoked responses showed that whereas initially a high proportion of them (40%) had an excitatory–inhibitory type of response (an increase in the number of spikes during the positive and a decrease during the negative component of FER), whereas the rest could be classed as of inhibitory–excitatory type, 1–2 h after injection of testosterone all neurons responded to stimulation only by inhibitory–excitatory and inhibitory types. In control animals 1–2 h after injection of peach oil the original ratio between neurons with the types of response described above remained virtually the same. These facts, and also data obtained by the writers previously [6], lead to the conclusion that immediate transformation of electrogenesis in AR of the hypothalamus during androgenization follows a regular pattern which is manifested as inhibition of

spontaneous and evoked spike electrogenesis arising against the background of synchronization of the integral electrogram and an increase in the amplitudes of EP.

Against the background of the electrophysiological changes described above in the tissues of the cerebral cortex, hypothalamus, adrenals, myocardium, liver, and also in the blood, 1 h after injection of testosterone, significant changes were observed in the catecholamine concentrations, the most demonstrative feature of which was a sharp rise in the DA concentration in all the tissues studied, and in particular in the hypothalamus. Meanwhile in the hypothalamus, cerebral cortex, adrenal medulla, and tissues of the peripheral organs (myocardium and liver) a sharp decrease was observed in the concentrations of NA and A. The parallel decrease in the concentration of the dopamine precursor dopa in the cerebral cortex, hypothalamus, myocardium, and liver, evidently arising because of increased transformation of dopa into DA, will be noted (Fig. 3). A rather different response was observed in the blood, where the concentration of all catecholamine fractions rose considerably, possibly due to their release from tissue depots. This release is evidently the result not so much of the effect of testosterone as a nonspecific response to nociceptive stimulation, for similar changes took place in the control experiments.

Under the influence of hyperandrogenization, reorganization of catecholamine synthesis and secretion probably takes place, in the direction of increased DA formation with parallel inhibition of the subsequent stages of NA and A biosynthesis. As a result considerable quantities of DA accumulate in the blood and in the tissues of various organs, accompanied by a sharp fall in the levels of A and NA. One specific effect of hyperandrogenization on the SAS is thus stimulation of processes responsible for predominance of dopamine regulation. The formation of the global response in the hypothalamic AR and, in particular, the amplitude of its negative component are evidently determined not so much by synchronization of spike discharges but rather by summation of postsynaptic potentials of simultaneously hyperpolarized neurons, which can be explained by predominance of inhibition processes. The possible cause of this is an excess of DA. This hypothesis is confirmed by the analogous electrophysiological and biochemical changes in the cerebral cortex and thalamus, where the amplitude of ER rises significantly after injection of testosterone, parallel with synchronization of the EEG in the ϑ-wave band.

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