SENSITIVITY OF VENTROMEDIAL HYPOTHALAMIC NEURONS TO THYROTROPHIN RELEASING HORMONE AND BRADYKININ: EFFECT OF IMMOBILIZATION STRESS

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Several investigations have shown that electrical or specific chemical stimulation of the ventromedial hypothalamus (VMH) initiates avoidance behavior and aggression and leads to the formation of emotional stress in immobilized animals [1, 4].

Neurons of DMH have been shown to contain larger quantities of thyrotrophin releasing hormone (TRH) than other brain structures [3]. Central injection of TRH initiates consummatory forms of food behavior, grooming, and orienting and investigative behavior in rats [11] and enhances motor activity [6]. Central injection of TRH causes an increase in the hypothalamic blood flow and the total cerebral blood volume and a rise of body temperature in rabbits but a fall in temperature in cats [10], evidence that TRH possesses antidepressant properties and indicates that it may be involved in the mechanism of positive reinforcement. According to data obtained by a number of workers [5, 8] an increase in the TRH concentration in hypothalamic structures is observed during emotional stress. At the same time, only 30% of neurons of the lateral and ventromedial hypothalamus change their firing pattern in response to microiontophoretic application of TRH into the perineuronal space [9].

It has been shown in our laboratory [2] that injection of bradykinin into the lateral ventricles of rabbits facilitates the negative emotional reactions to electrical stimulation of VMH and inhibits self-stimulation of the lateral hypothalamus. Central injection of bradykinin leads to the development of catalepsy, hyperglycemia, and hyperthermia, whereas peripheral application of bradykinin causes change in the latent periods of nociceptive responses in rabbits through activation of the opiate system [7].

Thus TRH and bradykinin are involved in the realization of negative emotions in animals with emotional stress. However, the concrete mechanisms of these influences have not been studied. The aim of the present investigation was to analyze changes in activity of VMN neurons in animals under the influence of microiontophoretic applications of TRH and bradykinin, under free behavior conditions and in emotional stress induced by immobilization.

EXPERIMENTAL METHOD

Experiments were carried out on seven mature male rabbits. Spike discharges of VMH neurons were recorded and TRH and bradykinin applied to VMH microiontophoretically by means of three-channel glass microelectrodes of pencil type. An aqeuous solution of TRH ("Serva," West Germany) in 5×10^{-4} M bradykinin solution ("Serva") was applied to single neurons. Microiontophoresis was carried out by means of an anodal current of 20-30 pA. The recording channel of the microelectrode was filled with 3 M KCl solution. Spontaneous neuronal spike activity and activity induced by neuropeptides (TRH and bradykinin) were recorded an analyzed with the aid of special programs and a microcomputer. Experiments were carried out on unrestrained animals and animals with emotional stress caused by gentle immobilization. The duration of immobilization stress varied from 30 min to 5 h. Altogether 23 VMH neurons were recorded in unrestrained rabbits and 22 neurons in rabbits in a state of immobilization stress.

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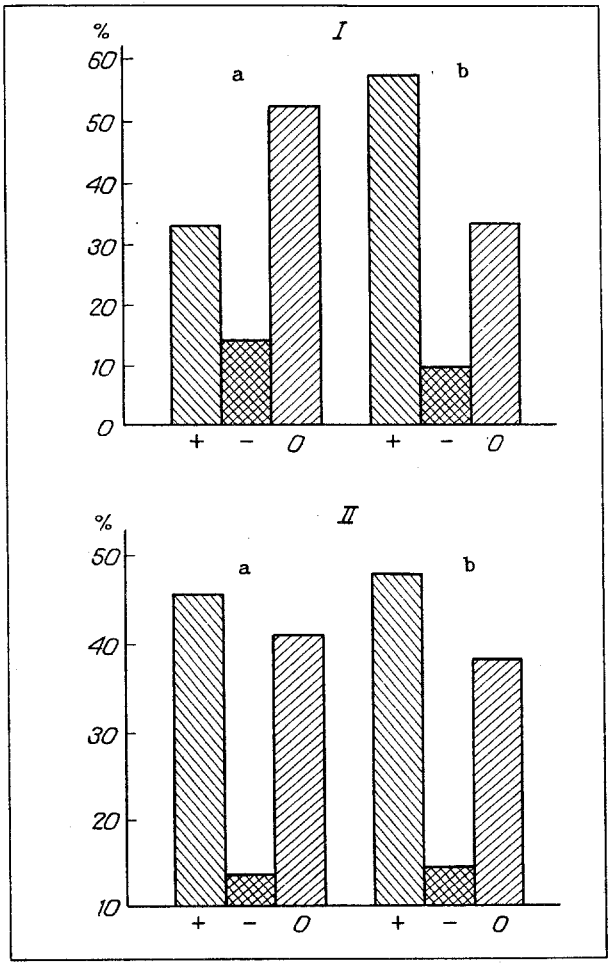

Fig. 1. Character of changes — +) activation, —) inhibition, 0) no response — in discharge frequency of VMH neurons in response to microiontophoretic application of TRH (I) and bradykinin (II) into the perineuronal space in unrestrained (a) and immobilized (b) rabbits.

EXPERIMENTAL RESULTS

Microiontophoretic applications of TRH to VMH neurons of unrestrained rabbits led to changes in the patterns of the original frequency parameters in 20 (87.0%) neurons. Three neurons (13.0%) were areactive to injection of TRH (Fig. 1, Ia). Twelve neurons (52.2%) reduced their firing rate in response to application of TRH, while preserving the original firing patterns, with predominance of interspike intervals within limits of 1-10, 100-300, and 10-20, 100-300 msec (Fig. 2a). Activation and regularization of spike activity were observed in eight nerve cells (34.8%) after application of TRH. After the end of TRH application by microiontophoresis, reorganization of firing patterns was observed in five neurons (21.7%): two neurons changed from bursting-grouped to continuous-arrhythmic, and three from single-arrhythmic to bursting-grouped spike activity. The character of the changes in irregularity of the spike trains of neurons, characterizing the steady state of spike activity, is shown in Table 1. In response to a single application of TRH to neurons of VMH, separate fragments of food consuming behavior were observed in the rabbits with a latent period of 60-120 sec after the end of iontophoresis: chewing, sniffing, etc. No changes were recorded in autonomic parameters (rise of body temperature, increased respiration rate, etc.), such as were observed after injection of TRH in rats [10].

Bradykinin, when applied into the perineuronal space of VMH cells, altered the spike activity of 19 (90.4%) of the 21 neurons recorded. Of this number, 12 neurons (57.1%) strengthened, whereas seven (33.3%) inhibited their spike activity. Two neurons (9.5%) were areactive toward bradykinin (Fig. 1, II, a). In ten reactive neurons (47.6%) bradykinin initiated a bursting-grouped type of activity with predominance of interspike intervals of 1-10, 100-300 msec (bursting-grouped type of activity).

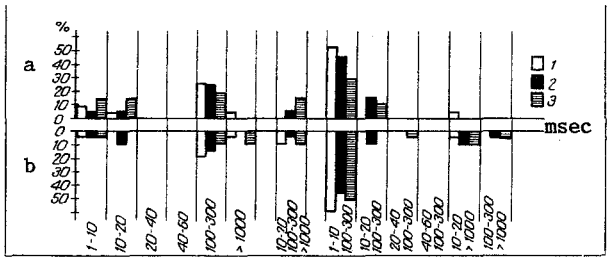

Fig. 2. Predominant interspike intervals in firing patterns of VMH neurons during microiontophoretic application (MIA) of TRH in unrestrained (a) and immobilized (b) rabbits. 1) Spontaneous activity, 2) MIA of TRH, 3) activity after MIA of TRH.

TABLE 1. Character of Changes in Average Parameters of Irregularity of Spike Discharges of VMH Neurons during Microiontophoretic Application of TRH and Bradykinin (Bk) in Unrestrained and Immobilized Rabbits ($M \pm m$)

Parameter	Unrestrained rabbit			
	increase in values	no change	decrease in values	change of values ambivalent
Number of neurons	$n=6$	$n=1$	$n=8$	$n=6$
Background	48,8±2,4	48,4±31,8	59,4±3,0	49,7±2,4
TRH	49,5±2,4	48,4±31,8	52,1±2,4	49,6±2,4
Background after TRH	49,5±2,4	48,4±31,8	50,6±2,8	49,7±2,4
Number of neurons	$n=6$	$n=0$	$n=8$	$n=7$
Bk	48,3±2,6	–	48,9±3,2	47,5±2,0
Background	42,3±2,6	–	52,3±3,2	51,4±2,0
Background after Bk	51,3±2,6	–	49,2±3,2	51,6±2,0
Immobilized rabbit (immobilization stress)				
Number of neurons	$n=12$	$n=0$	$n=10$	$n=0$
Background	44,1±3,5	–	53,6±3,2	–
TRH	51,77±3,5	–	48,5±3,2	–
Background after TRH	52,1±3,5	–	46,1±3,2	–
Number of neurons	$n=9$	$n=0$	$n=7$	$n=5$
Bk	38,6±3,2	–	49,5±2,6	50,4±2,0
Background	51,5±3,2	–	54,3±2,6	51,1±2,0
Background after Bk	55,6±3,2	–	47,4±2,6	50,5±2,0

After microiontophoretic application of bradykinin two neurons changed to a regular (continuous-arrhythmic) type of activity (Fig. 3a). In seven neurons, after application of bradykinin, single-arrhythmic activity was observed with predominance of interspike intervals of 100-300 msec and 1000 msec and longer. The character and structure of the patterns were changed in 18 (85.7%) neurons, and in nine of them (42.8%) the irregularity of the firing pattern was enhanced (Table 1). Stopping microiontophoresis of bradykinin in 11 neurons (52.4%), was followed after a latent period of 60-180 sec by restoration of the original level of spontaneous spike activity. In eight neurons (38.1%), after discontinuing microiontophoresis of bradykinin, prolonged (up to 15 min) changes in spike activity were recorded. No changes in behavior of the rabbits were observed at the time of the microiontophoretic applications of bradykinin to VMH neurons or after discontinuation of its application. The animals of this group, when studied under unrestrained conditions, were regarded as the control.

Emotional stress accompanying gentle fixation of the rabbits led to reduction of total activity of VMH neurons. The mean length of the interspike intervals in this case was increased by 12% ($p < 0.05$). The experiments showed that emotional stress created by gentle immobilization of rabbits, led to reorganization of the structure of the spontaneous firing pattern of VMH neurons compared with the control group. There was an increase in the number of neurons whose firing patterns were dominated by interspike intervals with a duration of 1-10, 100-300 msec. Microiontophoretic applications of TRH into the perineuronal space under these experimental conditions led to changes in the firing pattern of 19 (86.3%) of the 22 neurons tested (Fig. 1, I, b). Three neurons (13.6%) were areactive to microiontophoretic applications of TRH. No changes in the animals' behavior were recorded under these circumstances. In six neurons application of TRH was followed by a marked increase in the intensity of the initial firing pattern. Thirteen neurons restored the initial level of their activity 30-90 sec after the ending of TRH applications. Changes in temporal characteristics of the spike trains and in the character of patterns of interspike

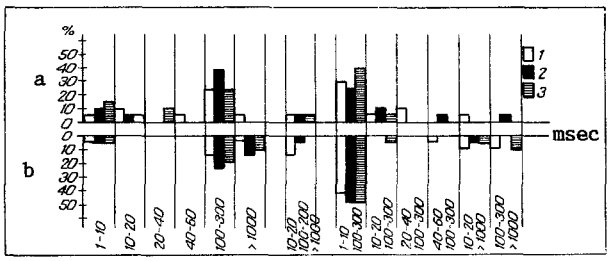

Fig. 3. Predominant interspike intervals in firing patterns of VMH neurons during microiontophoretic application of bradykinin in unrestrained (a) and immobilized (b) rabbits. 1) Spontaneous activity, 2) MIA of Bk, 3) activity after MIA of Bk.

intervals during microiontophoretic applications of TRH to the immobilized animals are shown in Fig. 2b. These results show that TRH had a twofold influence on the character of the spike discharge of VMH neurons. In 12 nerve cells (54.8%) an increase was noted in the mean values of irregularity of the spike discharges, whereas in 10 cells (45.5%), conversely, there was a decrease in these parameters, i.e., regularization of their spike activity was recorded (Table 1).

Microiontophoresis of bradykinin to VMN neurons of immobilized rabbits induced activation of the discharge in 11 nerve cells (52.3%) but inhibition in eight (38.2%) (Fig. 1, II, b). Three neurons (9.5%) were areactive to bradykinin. On microiontophoresis of bradykinin, there was an increase compared with the control animals (Fig. 3b) in the number of neurons with a bursting-grouped type of activity, and their firing pattern was dominated by intervals of 1-10, 100-300 msec (Fig. 3b).

Thus VMH neurons are characterized by specific sensitivity to TRH and to bradykinin. The experiments showed that emotional stress, associated with gentle immobilization of rabbits, is accompanied by changes in the spontaneous firing pattern of VMH neurons. Inversion of sensitivity of VMH neurons relative to TRH, but not to bradykinin, is observed.

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