ADRENOBLOCKER MODULATION OF THE ACTION OF PENTAGASTRIN ON LATERAL HYPOTHALAMIC NEURONS

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Attempts to explain the neurochemical mechanism of food motivational excitation have led to the concept of close interaction of neuropeptides and classical mediators [1] in the formation of patterns of neuronal activity characteristic of hunger [4]. This view is confirmed by the discovery of the coexistence and combined release of neuropeptides and mediators at the synaptic level [10] and of their mutual modulating action [1, 6].

The aim of this investigation was to study mechanisms of interaction between mediator and neuropeptide on single neurons of the lateral hypothalamus (LH) during administration of pentagastrin (PG) alone or followed by microiontophoretic adrenoreceptor blockade.

EXPERIMENTAL METHOD

Experiments were carried out on 17 unrestrained male Chinchilla rabbits weighing 2.5-3.5 kg. Spike discharges of LH neurons (AP 2, L 2, H 13-16) was recorded extracellularly by means of 4-barreled glass microelectrodes, whose tip had a resistance of 20-40 MΩ. The recording barrel was filled with 3 M NaCl solution, and the other three barrels with saturated solutions of prazosin (pH 6-6.5) and propranolol (pH 5.9-6.4) for microiontophoresis (MIP), and also with 0.1 M NaCl solution as a control for the effect of the current. MIP of propranolol and prazosin was carried out with an anodal current of 30-50 nA for 1 min, and the control MIP of NaCl with an anodal current of 15-25 nA for 30 sec. The PG used in the experiment was obtained from "Serva" (Germany), and dissolved in ammonia buffer and injected subcutaneously in a dose of 0.02 mg/kg [3]. PG was injected subcutaneously on the basis of data on permeability of the blood-brain barrier for this peptide [8]. The selective α_1-adrenoblocker prazosin and the nonselective β-adrenoblocker propranolol were dissolved in bidistilled water. The time course of single unit activity in LH during and after injection of PG and the subsequent microiontophoretic applications of the adrenoblockers was investigated. The experimental program was as follows: 1) recording spontaneous activity of LH neurons in previously fed rabbits; 2) subcutaneous injection of ammonia buffer; 3) subcutaneous injection of PG solution; 4) MIP of NaCl; 5) MIP of propranolol; 6) MIP of NaCl; 7) MIP of prazosin. Spike activity of LH neurons located along the microelectrode track was next recorded and MIP of NaCl and of the adrenoblockers was then carried out on them in the same order. The microelectrodes were inserted into the brain by means of a micromanipulator of original design, fixed to the skull of the experimental animal. An electrophysiological system adapted for unrestrained rabbits was used in the experiments. Unit activity was recorded on magnetic tape by means of a 4-channel 7003 measuring tape recorder (from Brüel and Kjaer, Denmark). Subsequent processing of the signal was carried out on an Apple II computer (USA). The program of analysis for each neuron included calculation of the mean interspike

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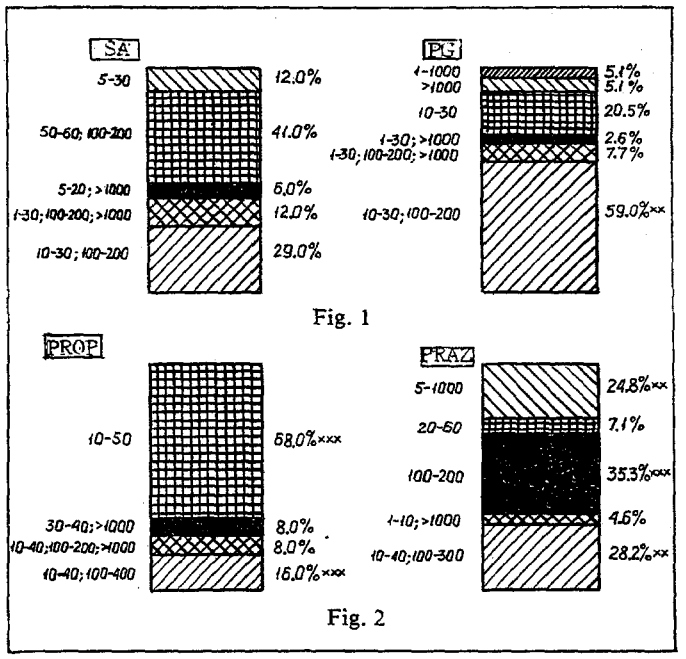

Figs. 1 and 2. Relative percentages of different types of distribution of ISI value on interval histogram with discontinuous and unequal scale. Numbers to left of columns denote dominant values of ISI in msec, numbers on right show fraction of neurons with the same type of distribution of ISI values (asterisks indicate significance of difference from values of preceding activity by Student's test). Explanation of abbreviations: SA) spontaneous activity, PG) after injection of pentagastrin, PROP) after MIP of propranolol, PRAZ) after MIP of prazosin.

interval, the coefficient of variation, and the mean firing rate [2]. A method of construction of histograms of the percentage distribution of interspike intervals (ISI) with discontinuous-unequal and logarithmic scales, and calculation of the coefficient of inequality of spike activity [1, 4] also was used.

EXPERIMENTAL RESULTS

Activity of 45 LH neurons, spontaneous activity of 17 neurons, and activity of 39 neurons after injection of PG, 25 neurons after MIP of propranolol, and 30 neurons after MIP of prazosin preceded by injection of PG, were investigated.

Depending on the character of their activity the neurons were divided into groups in accordance with the distribution of their ISI. The time course of the distribution of ISI after each procedure was studied. The main results of data processing are given in Figs. 1 and 2.

The dominant feature of spontaneous activity of the LH neurons of the satiated rabbits was a bimodal distribution with dominance of ISI in regions of the interval spectrum 50-60 and 100-200 msec (41%), which is characteristic of defensive motivation [4] (Fig. 1). Injection of the buffer solution caused no significant changes in unit activity. Spike activity of LH neurons 1-3 min after subcutaneous injection of PG underwent significant changes. A transition was observed to a "bursting" type of activity or shortening of ISI in the case of existing bursts. The largest group consisted of neurons with a bimodal distribution of ISI values within the regions of 1-30 and 100-200 msec (59%), characteristic of spike activity of LH neurons in a state of hunger [4] (Fig. 1). The second largest group consisted of neurons with a monomodal distribution of ISI in the region 1-30 msec (20.5%).

MIP of NaCl caused no significant changes. After MIP of propranolol a shift in the distribution of ISI toward shorter values was observed, and the largest group (68%) consisted of neurons with a monomodal distribution and with predominance of ISI in the region of 10-60 msec. This neuronal pattern is characteristic of the activity of LH neurons in animals receiving food reinforcement [4] (Fig. 2). After MIP of prazosin a change took place in the distribution of the intervals toward longer values. The numerically largest group consisted of neurons with a monomodal distribution and with predominance of ISI in the region of 100-200 msec (35%). Meanwhile there was a significant increase in size of the group of neurons with a polymodal distribution of ISI within the regions from 5 to 1000 msec (25%) (Fig. 2).

Evidence was thus obtained of a radical change in the pattern induced by the action of PG in a large proportion of LH neurons during adrenoreceptor blockade. In most cases this was expressed as a change from a bimodal to a monomodal distribution of ISI, with different characteristics, or a polymodal distribution (Fig. 2).

The data indicating that combined release of noradrenalin (NA) and fragments of cholecystokinin (CCK), such as PG (which is identical with the C-terminal fragment CCK5), and also simultaneous synthesis of NA and CCK in neurons in the mesencephalic region [10], and a similar effect following separate application of PG and NA to neurons of LH [1, 3], suggest interaction for PG and NA in the organization of the firing pattern characteristic of hunger. The concrete mechanisms of this phenomenon are not yet clear, but there is evidence of strengthening of the effect of CCK5 on account of cAMP [5], and on the dependence of binding of PG with receptors on cyclic nucleotides and K^+ and Ca^+ ions [7]. Activity of serine peptidases, which complete processing of CCK and utilize its fragments in synaptosomes [9], also is of definite importance, and their functional state may determine the existence of different CCK fragments, which in turn determines the specificity of the effects of CCK isolated from presynaptic endings.

On the one hand, this suggests the possibility of regulating conformation and expression of receptors for PG on account of noradrenergic mechanisms. On the other hand, it can be tentatively suggested that the noradrenergic system has an effect on the synthesis and activity of synaptic serine peptidases through cAMP and Ca-dependent mechanisms.

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