CHANGES IN SENSITIVITY OF SEPTO-HIPPOCAMPAL RESPONSES TO ACETYLCHOLINE DURING LONG-TERM POST-TETANIC POTENTIATION

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The phenomenon of long-term post-tetanic potentiation (LPTP) arising after one or two tetanizations of a presynaptic formation was first described during the recording of focal evoked potentials (EP) of the dentate fascia [10], and later of EP and cell discharges from the pyramidal layers of the hippocampus [4]. Because of the long duration (several hours or even days [12]) and certain other properties [2], this phenomenon is regarded as a simple model of the formation of memory or a conditioned-reflex connection.

One possible mechanism of LPTP may be a long-term increase in the efficiency of mediator action as a result of modifications to the sensitivity of the subsynaptic receptor membrane. The testing of this possibility is interesting because a similar mechanism has often been postulated to explain the formation of memory traces and of the conditioned reflex [6, 8, 11], and experimental data confirming the correctness of this suggestion have been published [7, 15].

The object of this investigation was to test the hypothesis that LPTP of septohippocampal responses, described previously [4], is based on an increase in sensitivity to acetylcholine (ACh). Data identifying ACh as the transmitter in the corresponding pathway [1] are sufficiently convincing. Sensitivity to this transmitter has been tested by measuring changes in EP during microiontophoresis of ACh [5].

EXPERIMENTAL METHOD

Experiments on unanesthetized rabbits were carried out by the method described previously [5]. Bipolar stimulating electrodes were inserted into the medial septal nucleus (AP = 1.5-3.0; L = 0; H = 5.0-8.5). Their more exact localization was verified by the appearance of a "population spike" — the fast negative component of EP, within the anterior segments of the dorsal hippocampus (AP = 1.0; L = 2.0; H = 7.5-8.3) in response to paired (interval 10-30 msec) pulses of equal or different amplitude (current 0.05-1.5 mA, duration 0.3-0.5 msec). Stimuli were applied in pairs separated by intervals of 2-5 sec. Multibarreled micropipets, the recording barrel of which was filled with 2M NaCl solution, were used for recording and for microiontophoretic injection of ACh. A 1M solution (pH 4.0) of acetylcholine chloride was used for injection. From the mean (of 10-15 presentations) amplitudes (A) of the "population spike" A_C and A_{ACh} in the control (blocking current 16 nA) and against the background of the action of ACh (current 20-80 nA) respectively, relative sensitivity to ACh was calculated before and after tetanization: $E = (A_{ACh} - A_C)/A_C$. The amplitude of the "population spike" was measured by the method suggested by Alger and Teyler [9]. Tetanization (frequency 10-20 sec^{-1}, duration 5-10 sec) was applied through the same stimulating septal electrodes. The value of LPTP was determined by the equation $P = (A - A_o)/A_o$, where A_o and A represent amplitudes of the "population spike" before and after tetanization, respectively.

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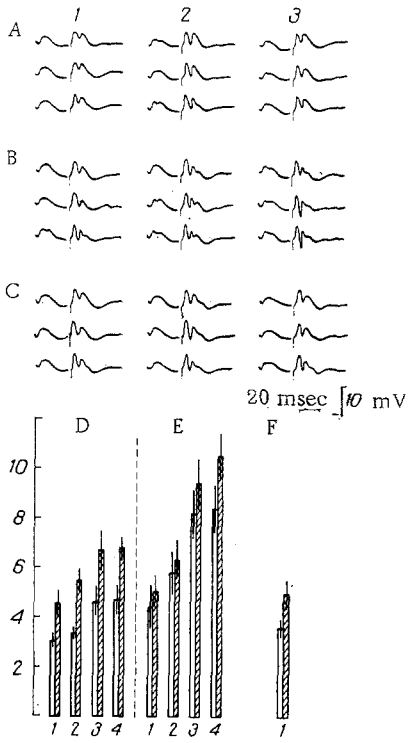

Fig. 1. Decrease in sensitivity of septo-hippocampal responses to ACh after tetanization. A) Focal responses arising in hippocampus to paired septal stimulation before tetanization; B, C) the same 19 and 79 min after septo-hippocampal tetanization. In A-C: 1) control responses before ACh application; 2) responses after microiontophoretic application of ACh; 3) the same immediately after end of ACh application. D-F) Changes in amplitude of population responses (to second stimulus in pair) recorded in same experiment before (D) and 19-64 min (E) and 70 min (F) after tetanization. Height of unshaded columns corresponds to mean amplitude (in mV) of population spikes before ACh application, calculated for 10-15 realizations. Shaded columns — the same after ACh application. Vertical lines show errors of means. Numbers along abscissa denote tests with different strengths of stimulation: 1) 1.50, 2) 1.65, 3) 1.80, 4) 1.95 mA.

EXPERIMENTAL RESULTS

The results of 12 experiments on six rabbits were analyzed. After 14 tetanizations, 60 testing stimuli of the same or different strengths were applied. An example of the increase in amplitude of the "population spike" described previously [5], which appeared in this experiment only in response to the second, stronger stimulus (Fig. 1A, 1), during microiontophoretic application of ACh (Fig. 1A, 2), is shown in Fig. 1A. The increase in A continued for several tens of seconds after the end of ACh application (Fig. 1A, 3). Comparison of the population spikes in Fig. 1A, 1 and Fig. 1C, 1 shows that tetanization induces LPTP. Iontophoresis of ACh against the background of LPTP also caused an increase in A, but the relative size of this increase was less than before tetanization. It will be clear from Fig. 2 that the first tetanization caused no significant changes in A (Fig. 2A and B), but nevertheless the value of E (Fig. 2C) was less than in the control. A second tetanization caused LPTP recordable throughout the experiment. E was reduced even more after the second tetani-

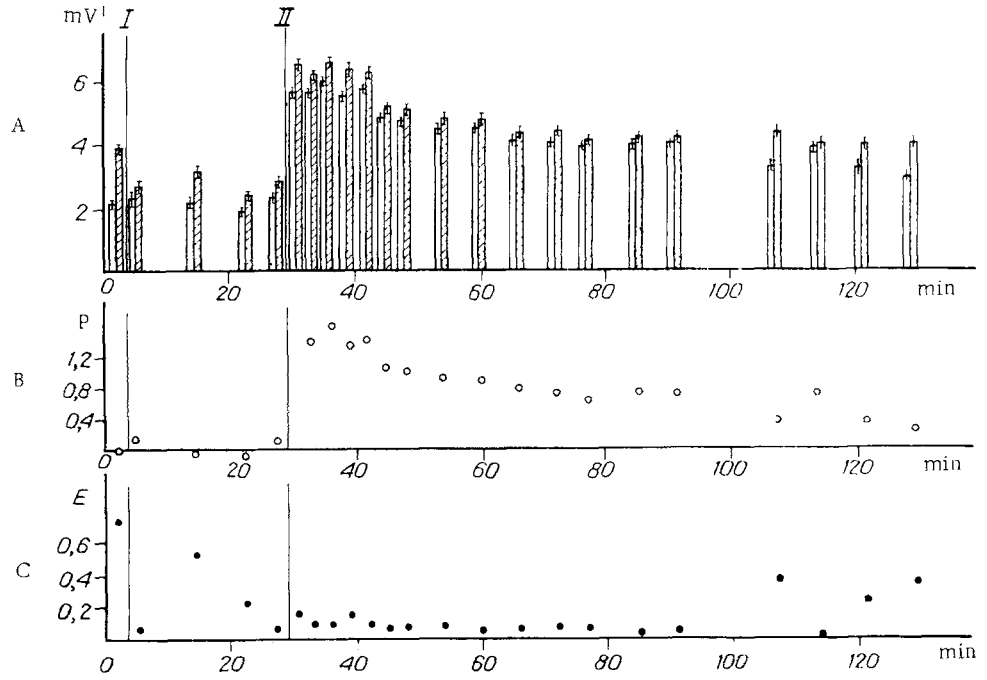

Fig. 2. Comparison of time course of LPTP and relative sensitivity of septo-hippocampal responses to ACh. I) 1st, II) 2nd tetanization. Abscissa in A-C, time (in min); ordinate: in A) amplitude of population spike before (unshaded columns) and during (shaded columns) ACh application, in B) relative value of potentiation, in C) relative sensitivity to ACh (see: "Experimental Method").

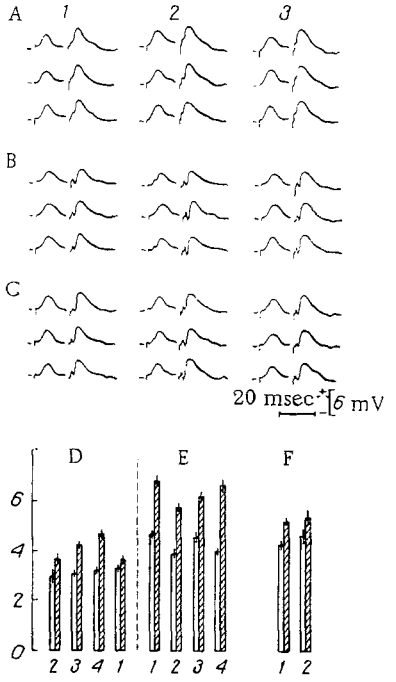

Fig. 3. Example of post-tetanic increase in sensitivity of septo-hippocampal responses to ACh. B, C) Hippocampal focal responses 2 and 31 min after tetanization; E, F) changes in amplitude of population spikes 2-26 min and 31-36 min after tetanization. Remainder of legend as in Fig. 1.

zation and remained lower than in the control throughout the experiment, i.e., there was significant negative correlation between it and the degree of potentiation (coefficient of correlation $r = -0.44$; $P < 0.05$). To discover whether the change in E was connected with a change (an increase during the development of LPTP) in A, in most experiments (11 of 12) different strengths of stimulation were used and responses with comparable A before (Fig. 1D, 4) and after (Fig. 1E, 1) tetanization were selected. As Fig. 1 shows, for responses so close in amplitude, sensitivity to ACh was appreciably reduced after tetanization.

LPTP (duration not below 5 min) was observed in only seven of the 12 experiments analyzed. In six of these experiments (seven tetanizations) E was reduced (on average from 0.46 ± 0.05 to 0.17 ± 0.06). In three of these experiments E was briefly (1-2 min) increased. A considerable increase in sensitivity to ACh, judging from responses evoked by stimuli of all intensities used, was observed in only one experiment (Fig. 3). Potentiation of E was particularly well marked in the first tests during the first few minutes after tetanization (Fig. 3E), after which E fell (Fig. 3F), to differ only a little from the control (Fig. 3D) despite continuation of LPTP.

In five experiments tetanization led to prolonged (over 5 min) post-tetanic depression. In two of them E was increased, and in three experiments it was reduced.

On average for all 47 tests with prolonged changes in A, a significant decrease (by Wilcoxon's nonparametric criterion) in E was observed from 0.57 (control) to 0.40 (after tetanization). Weak ($r = -0.41$) but statistically significant ($P < 0.01$) negative correlation was found between the value of LPTP and sensitivity to ACh. However, it must be emphasized once again that both a definite decrease (Figs. 1 and 2) and an increase (Fig. 3) in E could be observed in different experiments regardless of the sign of the post-tetanic changes.

On the whole the results do not confirm the hypothesis of the change in sensitivity to ACh as the mechanism lying at the basis of LPTP of septo-hippocampal responses. The decrease in sensitivity found in most experiments agrees with results obtained recently by Lynch et al. [14], who described a decrease in sensitivity of area CA_1 neurons to glutamate during LPTP in hippocampal slices. The decrease in sensitivity to mediators corresponds to the heterosynaptic depression [13] and prolonged hyperpolarization [3] described in some cases during LPTP. This long decrease in excitability and sensitivity to mediator, inhibiting responses to impulses arriving along nontetanized pathways, can be regarded as the analog of differential inhibition during conditioning.

In light of these results a more attractive hypothesis is that of a change in presynaptic efficiency as the main mechanism of LPTP.

LITERATURE CITED

1. O. S. Vinogradova, The Hippocampus and Memory [in Russian], Moscow (1975).
2. L. L. Voronin, Zh. Vyssh. Nerv. Deyat., 26, 705 (1976).
3. L. L. Voronin and I. E. Kudryashov, Zh. Vyssh. Nerv. Deyat., 29, 141 (1979).
4. L. L. Voronin, I. E. Kudryashov, and S. V. Ioffe, Dokl. Akad. Nauk SSSR, 217, 453 (1974).
5. R. G. Kozhedub, S. N. Kozhechkin, and L. L. Voronin, Zh. Vyssh. Nerv. Deyat., 28, 411 (1978).
6. R. I. Kruglikov, Izv. Akad. Nauk SSSR, Ser. Biol., No. 6, 902 (1978).
7. R. I. Kruglikov, O. Kh. Koshtoyants, and V. B. Val'tsev, Zh. Vyssh. Nerv. Deyat., 27, 989 (1977).
8. E. I. Rozanov, Usp. Sovrem. Biol., 76, 447 (1973).
9. B. E. Alger and T. J. Teyler, Brain Res., 110, 463 (1976).
10. T. V. P. Bliss and T. Lomo, J. Physiol. (London), 232, 331 (1973).
11. J. A. Deutch, Science, 174, 788 (1971).
12. R. M. Douglas and G. V. Goddard, Brain Res., 86, 205 (1975).
13. G. S. Lynch, T. Dunwiddie, and V. Gribkoff, Nature, 266, 737 (1977).
14. G. S. Lynch, V. K. Gribkoff, and S. A. Deadwyler, Nature, 263, 151 (1976).
15. J. Schmidt, H. Rüthrich, and E. Kammerer, in: Neurobiological Basis of Memory Formation, Vol. 1, Berlin (1974), p. 262.