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Pre- and Postsynaptic Mechanisms of the Deprivation Potentiation of Neuron Population Responses in Rat Hippocampal Field CA1

V. A. Popov

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Studies using in vitro rat hippocampal slices addressed the mechanism of deprivation potentiation (DeP) of neuron population responses in field CA1 developing as a result of prolonged (60 or 120 min) cessation of rare (0.05 Hz) test stimuli of Schaffer collaterals (SC). The development of DeP was found to involve two independent mechanisms: pre- and postsynaptic, responsible for induction of the short-term and long-term components of DeP, respectively. These studies showed that the interaction of DeP and long-term post-tetanic potentiation (LTP) is competitive in nature, providing evidence of similarity in the mechanisms of the long-term phase of DeP and the phase of LTP associated with protein phosphorylation. Our investigation showed that the Ca^{2+}-dependent mechanism of induction of the postsynaptic component of DeP does not involve NMDA receptors, but does involve purine P2 receptors. A common curve for the relationship between the efficiency of synaptic transmission and the use/non-use of synapses including DeP, long-term depression (LTD), and LTP zones is presented.

Keywords: deprivation potentiation, hippocampal slices, population spike, antidromal stimulation, NMDA receptors, MK-801, purine P2 receptors, PPADS.

One form of synaptic plasticity, associated with changes in the nature of afferentation and distinct from such well known forms as LTP and LTD [Bliss and Collingridge, 1993; Bliss and Lømo, 1973; Lynch et al., 1977], is deprivation potentiation (DeP), which consists of an increase in the efficiency of synaptic connections as a result of prolonged non-use (deprivation). Experiments on living rat hippocampal slices testing connections between Schaffer collaterals (SC) and field CA1 neurons showed that cessation of rare (0.05 Hz) test stimuli for 4 h led, after stimulation was restarted, to a significant increase in the amplitude of population spikes [Popov, 1994]. Analogous results were obtained in chronic in vivo experiments in rats during drug-induced sleep on testing the connections of the medial perforant bundle (MPB) and neurons in the dentate fascia (DF) of the hippocampus [Popov and Markevich, 1999]. The magnitude of DeP was proportional to the duration of deprivation (10–120 min), reaching values comparable with that of LTP; testing showed that DeP persisted for more than an hour [Popov and Markevich, 2001]. Our studies demonstrated the involvement of extracellular Ca^{2+} and intracellular Ca^{2+} depots and protein kinase C (PKC) in the mechanism of DeP [Popov and Markevich, 2001, 2014]. The pattern of DeP revealed two phases with presumptively different origins: an initial transient "peak" (10–12 min) and a longer-lasting "plateau" (more than 1 h) [Popov and Markevich, 2014]. The aim of the present work was to study the nature of the initial transient phase of DeP, the mechanism of induction of DeP, and the interaction of DeP and LTP.

Methods

Experiments were performed on living Wistar rat (150–200 g) hippocampal slices prepared from rats anesthetized with ether before decapitation. Slices of thickness 400 μm were placed in a recording chamber with shallow immersion in artificial cerebrospinal fluid (aCSF). This solution contained 124 mM NaCl, 3.0 mM KCl, 26 mM $NaHCO_3$,

Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia;
e-mail: v-lad-i-mir@yandex.ru.

1.25 mM K_2HPO_4, 2.2 mM $CaCl_2$, 1.8 mM $MgCl_2$, and 10 mM D-glucose; the solution was saturated with a gas mix consisting of 95% O_2 and 5% CO_2 at a temperature corresponding to that used in the recording chamber (32°C); pH was 7.4; flow rate was 0.9 ml/min. Slices were incubated for 2 h before stimulation started.

Total population spikes (PS) were recorded in the pyramidal layer of hippocampal field CA1 using glass microelectrodes filled with 2 M NaCl solution. Electrode resistance was 3–5 MΩ. PS amplitude was measured from the first positive peak to the negative peak of the response. Monopolar stimulating electrodes (electrolytically sharpened tungsten wires 100 μm thick, coated with Viniflex lacquer) were established in the area of SC in the mid part of the stratum radiatum of hippocampal field CA1. In experiments using antidromal stimulation, an additional stimulating electrode was placed symmetrical to the recording electrode in the axon area (the alveus). Test stimulus amplitude was selected such that PS amplitude was about 30% of the maximum; stimulus duration was 100 μsec; test stimulus frequency was 0.05 Hz.

The DeP induction procedure in the experiments consisted of interrupted test stimulation for 60 or 120 min, after which stimulation was restarted; in controls, test stimulation was performed throughout the experiment.

In a separate series of experiments, stimulus amplitude was decreased during the first 14–15 min after deprivation either to the initial threshold value with a gradual increase to the test level or to a value at which PS amplitude was no greater than the baseline response level; further testing was with the initial stimulus parameters.

LTP was induced by tetanization with three volleys of four spikes at the test amplitude with a frequency of 100 Hz, with an intervolley interval of 200 msec.

Studies used the selective noncompetitive NMDA receptor antagonist (5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801) (MP Biomedicals, France) at a concentration of 30 μM and the nonselective purine P2 receptor antagonist pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) (Sigma-Aldrich, USA) at a concentration of 20 μM. Substances were initially dissolved in water (concentrated stock solution); stock solution was diluted in aCSF to the final concentration a few minutes before switching the flow system. MK-801-containing solution was added to the chamber 20 min before deprivation started. PPADS-containing solution was delivered during 60-min deprivation and test stimulation was restarted at the beginning of washing to remove this solution.

The responses recorded were averaged for 10 stimulus presentations. The mean response to the last 10 predeprivation stimuli was taken as 100%. Plots were made and statistical analysis of results was performed using Student's *t* test and Marquardt nonlinear parameter assessment (SigmaPlot 12.0 and Statistica 7).

Experiments were performed in compliance with the humanitarian principles laid out in the Directives of the European Community (86/609/EC) and were approved by the Medical Ethics Committee in accordance with the position of the Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences on work with experimental animals.

Results

The previously demonstrated suppressing influence of preliminary induction of LTP on the development of DeP [Popov and Markevich, 2001] provided grounds for the suggestion that these two forms of plasticity may have similar mechanisms operating in a competitive fashion and that this influence should be mutual. Figure 1, *A* shows the experimental protocol: initial stimulation (measurement of baseline responses), 120-min deprivation, brief testing of DeP, selection of the new stimulus amplitude (normalization of responses to the initial level), tetanization, and testing of LTP with the new stimulus parameters. Figure 1, *B* shows the overall data. In the experiment, as compared with controls (tetanization during the same period of time after continuous test stimulation), preliminary induction of DeP led to suppression of the prolonged phase of LTP: immediately after tetanization (first averaging period), differences between mean PS amplitudes in the experiment and controls were statistically insignificant (178.3 ± 7.1%, $n = 10$, and 211.0 ± 13.6%, $n = 11$, $p > 0.05$), though by 5 min the difference was significant ($p < 0.05$ in the second and third time periods, then $p < 0.01$). Thus, the suppressing action of prior induction of DeP on the development of LTP supports the suggestion that the mechanisms of development of the long-term phases of LTP and DeP are similar and in competition.

Theoretically, two scenarios for the development of DeP are possible. In the first, induction occurs during synaptic rest and reinitiation of stimulation demonstrates DeP and its persistence. The similarity of the mechanisms of LTP and DeP, their Ca^{2+} dependence, and the high level of the initial peak of DeP provide grounds for suggesting an LTP-like, NMDA-mediated mechanism for the development of DeP during maximal synaptic activity after reinitiation of stimulation. With the aim of testing this hypothesis, we conducted two series of experiments, eliminating the prerequisite for activating NMDA receptors or blocking them. The aim of the first series was to cancel the initial increase in the response. In the experiment (Fig. 2, *A*), during the first 15 min after a 60-min period of deprivation (the duration of the initial DeP peak was 10–12 min [Popov and Markevich, 2014]), stimulation was with a constantly increasing amplitude from the initial near-threshold value to the test value; subsequent test stimulation displayed the pattern of the DeP plateau phase. A somewhat different experimental scheme is shown in Fig. 2, *B*: a 60-min period of deprivation was followed by weakened stimulation for the first 14 min, using current impulses generating responses no larger than baseline responses, after which the test stimulation returned

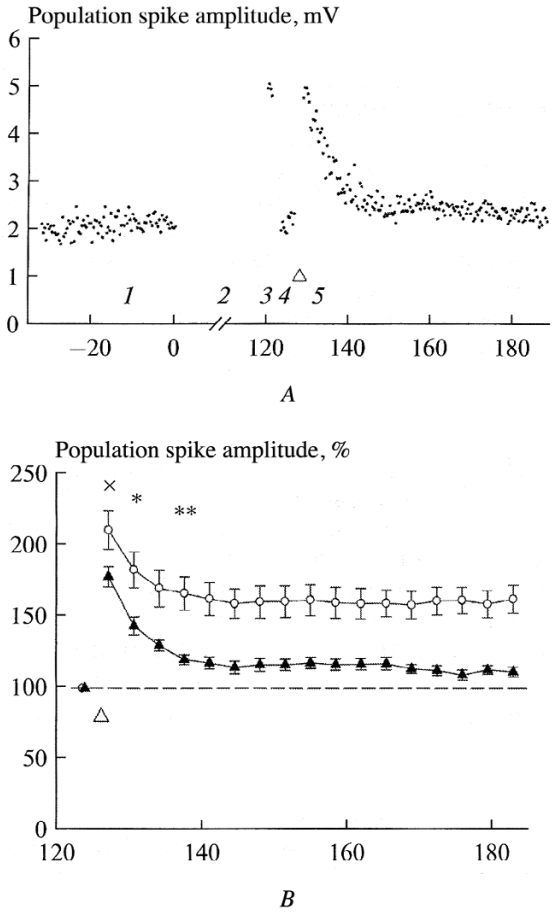

Fig. 1. Preliminary induction of deprivation potentiation (DeP) suppresses the development of the long-term but not the short-term phase of long-term post-tetanic potentiation (LTP). *A*) Experimental protocol; *1*) initial test stimulation; 2) 120-min cessation of stimulation (deprivation); *3*) testing of DeP; *4*) new test stimulation at which the amplitude of population spikes (PS) was decreased to the initial response level; *5*) testing of LTP (tetanization is shown by the light triangle). The abscissa shows time, min; the ordinate shows PS amplitude, mV. *B*) Overall data: black triangles show the experiment (n = 10), and light circles show controls (tetanization at the corresponding time after continuous test stimulation) (n = 11). The abscissa shows time, min, and the ordinate shows mean PS amplitude, %; tetanization is shown by the white triangle; the dotted line shows the baseline response level taken as 100% (mean PS amplitude for the last 10 pre-tetanization stimuli). Values are shown as mean ± error of mean; statistically significant differences between values in experiments and controls, Student's t test: **,*significant ($p < 0.01$, $p < 0.05$, respectively), ^{x}not significant ($p > 0.05$).

to initial parameters. The similarity in the rate of decline of responses in the experiment with weakened stimulation (black triangles) and the corresponding period of DeP in controls (standard testing, light circles) is evidence of a decrease in the initial strengthening of responses in the experiment. After returning to test stimulation parameters, the experiment showed a pattern of responses similar to DeP in the corresponding period in controls: pairwise comparison of averaged PS amplitudes in the experiment (n = 11) and controls (n = 14), starting from the fifth time period, demonstrated the absence of any significant difference ($p > 0.1$). In the second series of experiments (Fig. 3), involvement of NMDA receptors in inducing DeP was tested by performing 60-min deprivation on the background of blockade of NMDA receptors with MK-801 (30 μM). In the experiment, MK-801 had no significant influence on the development of DeP: comparison of mean amplitudes after deprivation in the experiment on the background of MK-801 (Fig. 3, black triangles, n = 13) and in normal conditions (Fig. 2, *B*, white circles, n = 14) showed that the differences between curves were within the range of random variation ($p > 0.05$). In controls (constant stimulation), MK-801 had no significant influences on response amplitude (Fig. 3 – white circles, n = 7).

Thus, the results of two series of experiments showed that induction of DeP had a mechanism differing from that of induction of LTP, triggered not during the peak of synaptic activity but during the period of synaptic rest and not involving NMDA receptors.

We have previously suggested that the initial DeP peak is presynaptic in nature [Popov and Markevich, 2001, 2004]. In experiments performed to test this hypothesis (Fig. 4), antidromal stimulation of the corresponding axonal area (the state of postsynaptic activity) was performed during 60-min deprivation (state of presynaptic rest). Antidromal stimulation (0.05 Hz) during deprivation had a suppressing effect on the development of DeP: pairwise comparison using Student's test showed that the mean amplitudes of PS in the experiment (Fig. 4; n = 9) and in normal conditions (Fig. 2, *B*, white circles; n = 14) identified significant differences ($p < 0.01$). The occurrence of an initial peak in responses due solely to presynaptic rest is evidence of its presynaptic nature. The incomplete return of responses to the baseline level may be linked with incomplete coincidence of the zones of presynaptic and antidromal activation. The difference between the peak amplitude and the new level of response stabilization (averaged PS amplitude in the period 77–94.5 min) allowed the magnitude of the presynaptic increase relative to the baseline response level (100%) to be evaluated: 138.3 – 111.1 = 27.2%. An analogous calculation for DeP (the differences between the maximum value of the peak and the subsequent plateau level in the corresponding time interval) gave a similar result: 173.1 – 147.6 = 25.5%. Nonlinear assessment of the two curves using the Marquardt method also showed that they had an identical range and rate of decline in the first phase (indicating a common pattern), but had different asymptotic levels of stabilization in the second phase. Thus, the whole pattern of DeP can be represented as the sum of a transient presynaptic component and a long-term postsynaptic component, which develop independently during synaptic rest.

The non-involvement of potential-dependent NMDA receptors in the mechanism inducing the postsynaptic component of DeP suggests that P2 purine receptors may play an alternative role in this process. Ionotropic P2X receptors

are known to have relatively high calcium permeability during the resting potential of the cell, while activation of metabotropic P2Y receptors promotes release of Ca^{2+} from the endoplasmic reticulum (ER) [Pankratov et al., 2009]. To test this hypothesis, experiments with 60-min deprivation were performed with solution containing the purine P2 receptor antagonist PPADS (20 μM). Test stimulation was reinitiated immediately after switching the flow system to normal solution. In these experiments, PPADS suppressed the prolonged plateau phase (the postsynaptic component of DeP), without having any significant effect on the development of the initial peak (the presynaptic component of DeP): mean PS amplitudes in the experiment (Fig. 5, black triangles) and controls (Fig. 5, white circles) immediately after reinitiating stimulation were 137.5 ± 3.9% ($n = 9$) and 103.5 ± 2.4% ($n = 7$) (significant difference, $p < 0.01$), while about 12 min later (the time taken for the presynaptic increase to decline) these values were 116.5 ± 4.0% and 106.0 ± 1.2%, respectively (the difference was not statistically significant, $p > 0.05$, then $p > 0.1$). Comparison of the plots for experiments with PPADS (Fig. 5, black triangles) and with antidromic stimulation (Fig. 4) showed that there were no significant differences ($p > 0.1$), providing evidence that PPADS has no effect on the presynaptic component of DeP. Thus, it follows from these experiments that purine P2 receptors are involved in the mechanism of induction of long-term postsynaptic component of DeP but not the transient presynaptic component.

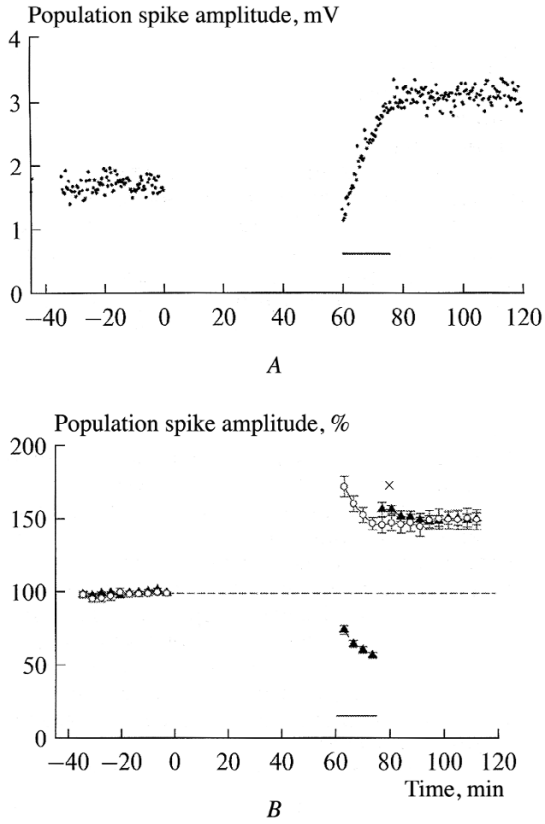

Fig. 2. Weakening of stimulation during the "peak" phase has no effect on the development of the "plateau" phase of DeP after 60-min deprivation. *A*) Experiment with gradual increase in stimulus amplitude during the first 15 min after deprivation from the near-threshold value to the test value, then test stimulation; *B*) experiments with decreased stimulus amplitude (PS amplitude below baseline response level) during the first 14 min after deprivation, then test stimulation; black triangles show the experiment ($n = 11$), and white circles show controls (standard testing of DeP) ($n = 14$). The bar shows the period of weakened stimulation. The abscissa shows time, min; the ordinate shows PS amplitude, mV (*A*) or mean PS amplitude, % (*B*).

Discussion

Our previous studies on living hippocampal slices with recording of PS of neurons in field CA1 on stimulation of SC, and also in chronic experiments on animals in the state of anesthetic sleep (recording of PS from neurons in the DF during stimulation of MPB) showed that prolonged deprivation (cessation of stimulation for periods ranging from 10 min to 4 h) induced marked potentiation of responses which could last more than an hour, which we termed deprivation potentiation [Popov, 1994; Popov and Markevich, 1999, 2001]. The suppression of the development of DeP obtained with a PKC blocker and prior induction of LTP [Popov and Markevich, 2001] provided grounds for suggesting that the mechanisms of DeP and the PKC-dependent phase of LTP associated with protein phosphorylation were similar [Frey et al., 1995; Reymann et al., 1988; Reymann, 1993]. Competitive suppression of two related mechanisms can occur on condition that the strength of the suppressive factor is comparable to (or greater than) that of the suppressed factor, and also that the latter occurs close in time to the former. To verify the reverse influence of DeP on LTP, we used experimental conditions in which potentiation of both was of essentially equal magnitude, DeP being "strengthened" (the deprivation period was increased from 60 to 120 min) and LTP was "weakened" (the number of stimulus volleys was decreased from five to three). In these experiments, prior induction of DeP had a suppressive influence on the development of the prolonged phase of LTP without having any significant effect on the short-term phase, which is evidence for a competitive interaction between DeP and LTP and supports the hypothesis that the mechanisms of the long-term phase of DeP and the phases of LTP linked with protein phosphorylation are similar.

This similarity in the mechanisms of DeP and LTP, their dependence on Ca^{2+} and PKC, the high level of initial postdeprivation responses, and the existence of data showing increases in the NMDA component of population EPSP after 60-min interruption of stimulation [Niu et al., 1999] provide grounds for suggesting an LTP-like NMDA-dependent mechanism of induction of DeP. To test this hypothesis, two series of experiments were performed. In the case of development of DeP via an NMDA-dependent mechanism, elimination of the initial presynaptic increase in the response or deprivation in the presence of an NMDA receptor antagonist (MK-801) should hinder the develop-

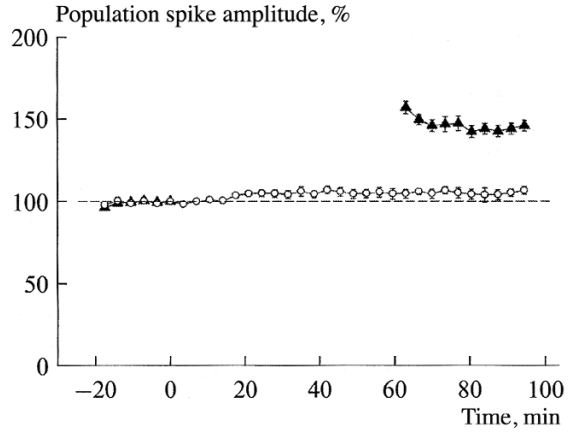

Fig. 3. NMDA receptors do not have a role in the mechanism of induction of DeP. Addition of the selective noncompetitive NMDA receptor blocker MK-801 to the solution (30 μM) had no effect on the development of DeP. Black triangles show the experiment (n = 13); white circles show controls (n = 7).

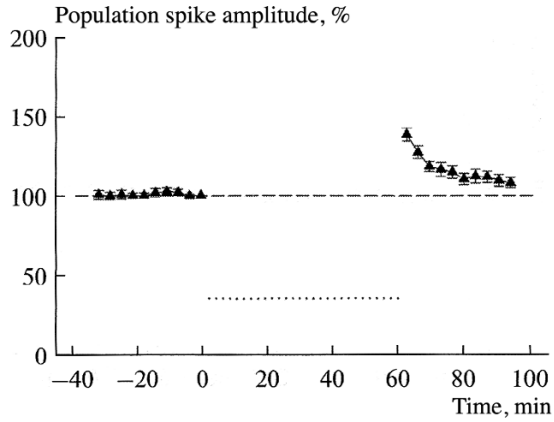

Fig. 4. Short-term facilitation of PS results from specifically presynaptic rest. Antidromal stimulation (postsynaptic activity) during 60-min deprivation (presynaptic rest) suppresses the development of the long-term plateau phase of DeP but not the initial short-term peak (n = 9); dots show antidromal stimuli.

ment of DeP. Negative results from both series of experiments showed that the mechanism of induction of DeP was not NMDA-dependent and developed not at the time of the peak in synaptic activity, but during synaptic rest. Despite the non-involvement of NMDA receptors in the mechanism of induction of DeP, it is too early to exclude the reverse situation – an effect of DeP on NMDA receptors.

Previous studies identified two phases in the pattern of DeP: an initial Ca^{2+}-independent peak (apparent both at a reduced extracellular Ca^{2+} concentration and in the presence of the penetrating Ca^{2+} chelator BAPTA-AM) and a prolonged Ca^{2+}-dependent plateau [Popov and Markevich, 2014]. In studies of the nature of the initial short-term DeP peak, antidromic stimulation (the state of postsynaptic activity) was applied during a 60-min break in orthodromic test stimulation (the state of presynaptic rest), at the same frequency, with the result that reinitiation of test stimulation was followed by suppression of the DeP plateau phase which marked initial facilitation of the response. These experiments showed that the origination of the initial short-term DeP peak involves a presynaptic mechanism presumptively associated with an increase in transmitter release.

Thus, three stages can be seen in the history of DeP: an initial stable state (baseline response level), induction of DeP during deprivation, and persistence of DeP on reinitiation of stimulation. The induction of DeP involves two different mechanisms: a Ca^{2+}-independent presynaptic and a Ca^{2+}- and PKC-dependent postsynaptic mechanism presumptively associated with protein phosphorylation. The two phases in the pattern of DeP reflect *a*) the sum of the transient presynaptic facilitation and the long-term postsynaptic potentiation with a ratio of about 1:2 (the initial "peak" phase) and *b*) persistence of long-term postsynaptic potentiation (the plateau phase).

The fact that Ca^{2+}-dependent induction of DeP develops not at the moment of peak synaptic activity but during the period of synaptic rest and without the involvement of NMDA receptors provided grounds for suggesting that the function of the calcium gates in the mechanism of induction of DeP is performed by non-glutamate ionotropic receptors. Purine P2X receptors constitute a family of ligand-activated channels, and their natural ligand is adenosine triphosphate (ATP). ATP molecules are known to be not only the main intracellular energy source, but also a widely distributed, evolutionarily ancient extracellular transmitter [Burnstock, 2013]. Despite the low concentration in the environment, natural ATP release is physiologically significant and can influence various long-term cellular responses [Burnstock and Verkhratsky, 2012]. Among the unique properties of P2X receptors is that they are the main Ca^{2+} channels during the membrane resting potential when NMDA receptors are blocked by Mg^{2+} (in contrast to NMDA receptors, Ca^{2+} entry into cells via P2X receptors, which is maximal at the resting potential, decreases on membrane depolarization), along with their ability to interact with other receptors and modulate synaptic plasticity. However, activation of metabotropic G-protein-mediated P2Y receptors evokes a cascade of intracellular reactions ending with release of Ca^{2+} from the ER and triggering the mechanism activating PKC [Pankratov et al., 2009]. These properties of P2X and P2Y receptors, making up the class of P2 purine receptors, correlate with our data on the involvement of extracellular and intracellular depot Ca^{2+} and PKC in the mechanism of development of DeP [Popov and Markevich, 2001, 2014], which provided grounds for suggesting the involvement of these receptors in the development of DeP.

In experiments, the standard procedure for induction of DeP using 60-min deprivation was performed on the background of one of the mist widely used antagonists of P2 re-

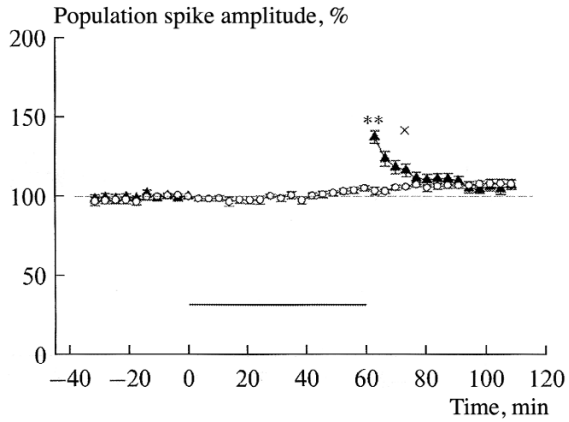

Fig. 5. Involvement of P2 purine receptors in the mechanism of DeP. Addition of the P2 purine receptor antagonist PPADS (20 μM) to the solution during 60-min deprivation suppresses the development of the long-term phase of DeP without affecting the development of the short-term presynaptic facilitation of PS. Black triangles show the experiment ($n = 9$); white circles show controls ($n = 7$); the horizontal bar shows addition of PPADS. For further details see caption to Fig. 1.

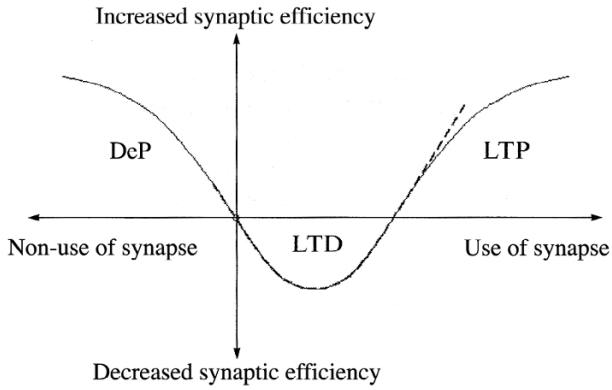

Fig. 6. Overall curve of the relationship between synaptic efficiency and synapse "use." Areas corresponding to LTP, LTD, and DeP are shown. The intersection of axes corresponds to synaptic efficiency with "normal" presynaptic activity (baseline response level at low-frequency stimulation). The dotted line shows Maiorov's curve.

ceptors, PPADS (20 μM). These experiments showed that PPADS, which has no notable influence on the presynaptic component, suppresses the postsynaptic component of DeP, which is evidence for the involvement of P2 receptors in inducing the long-term phase of DeP, which is postsynaptic in nature.

The great diversity of P2 receptors, considering the seven subunits of the P2X receptor family ($P2X_1$-$P2X_7$) and eight subunits of the P2Y family ($P2Y_{1,2,4,6,11,12\text{-}14}$), the species specificity of their distributions in the CNS, and the different activity of PPADS, makes it impossible to determine their precise functional role. Analysis of the possible participation of one or another P2 receptor subunit in the mechanism of induction of DeP is based on reviews [Ziganshin, 2005; Burnstock and Verhratsky, 2012; Köles et al., 2011; Pankratov et al., 2009]. Of the P2X receptor family, the most likely candidate is the $P2X_2$ subunit: this has the greatest representation in the CNS, including the hippocampus; it functions as a Ca^{2+} channel; formation of heterodimers with $P2X_6$ subunits ($P2X_{2/6}$) leads to an increase in Ca^{2+} permeability (colocalization with glutamate AMPA receptors has been demonstrated for both homopolymers); PPADS is an antagonist. $P2X_1$ receptors, which have even greater Ca^{2+} permeability, have similar characteristics, though they rapidly lose sensitivity to ATP. It can be suggested that when activated simultaneously with $P2X_2$ receptors, $P2X_1$ receptors play a triggering role in the mechanism of induction of DeP. The absence of any reports on the localization of $P2X_5$ receptors in the hippocampus does not allow any conclusion to be drawn regarding this subunit, as others are not candidates for a role in the induction of DeP: $P2X_3$ are specifically expressed in sensory neurons, $P2X_4$ are insensitive to PPADS, $P2X_6$ homomers do not function in the brain, and $P2X_7$ receptors are activated by very high ATP concentrations and are relatively insensitive to PPADS.

In the P2Y receptor family, only three P2Y subunits ($P2Y_{1,2,13}$) are purine-sensitive and mediate Ca^{2+} efflux from the ER. Of these, the most likely candidate for involvement in the mechanism of induction of DeP is the $P2Y_1$ subunit: it is present in the hippocampus in rats; adenosine diphosphate (ADP), formed when ectonucleotidases act close to sources of ATP, is an agonist; PPADS is a powerful antagonist. For $P2Y_{13}$, there are no data showing its presence in the hippocampus; PPADS is a weak antagonist of $P2Y_2$ receptors present on rat hippocampal pyramidal neurons. Other receptors either do not mediate release of Ca^{2+} from the ER ($P2Y_{6,11,12}$ receptors) or are pyrimidine-sensitive ($P2Y_{4,14}$ receptors, ATP being a competitive antagonist of $P2Y_4$ receptors).

In the nervous system, ATP is produced by both neuronal and glial cells. Of the possible routes of release of ATP (vesicular release, diffusion through pores in the plasmalemma, and active transport), cotransmission with glutamate and diffusion through potential-dependent anion channels can be excluded from a role in inducing DeP, because one condition for the induction of DeP is synaptic rest. As ATP is as "short-range" molecule because of the presence of ectonucleotidases, it can be suggested that ATP-conducting channels formed by pannexins present on the postsynaptic sites of hippocampal pyramidal cells [Thompson et al., 2008] may be "close sources" of ATP for P2 receptors. The possibility that P2X receptors might be activated not only by exogenous, but also by endogenous ATP [Pankratov et al., 2009] is also of interest.

The fact that one pathway for ATP release may consist of diffusion due to impairment of cell membrane integrity, which is unavoidable during preparation of slices, raises

the question of whether DeP is "pathological plasticity," or whether this potentiation is "physiological." The latter view may be supported by the induction of DeP in chronic in vivo experiments on animals in a state of anesthetic sleep and by the pattern of development of DeP, whose three stages are related to the beginning and end of deprivation: 1) the initial stable state, 2) induction of DeP, which has pre- and postsynaptic components, and 3) the stage of persistent DeP with short-term and long-term phases. In the case of pathological increases in ATP in the surrounding medium, changes in responses must be continuous in nature and must not be linked to the beginning or end of deprivation (cases of destabilized responses – an indicator of unsatisfactory slice viability – may provide an illustration of pathological changes). However, the possibility that DeP may be an early mechanism of the well-known clinical phenomenon "denervation hypersensitivity" cannot be excluded [Scharpless, 1964]. Countering Eccles' view [Eccles, 1961] that increased synaptic transmission efficiency occurs as a result of prolonged increases in synapse use, Scharpless, working from clinical data (sequelae of denervation, long-term deprivation), put forward the hypothesis that increased synaptic efficiency occurs as a result of a functional reduction in synapse use. The discovery of the phenomenon of LTP [Bliss and Lømo, 1973] was consistent with Eccles' hypothesis, while the phenomenon of LTD [Lynch et al., 1977] did not contradict Scharpless' views. Attempts to reconcile these opposite views were made by Maiorov in his "modified synaptic use hypothesis," which describes bidirectional changes in synaptic efficiency depending on the nature of presynaptic activity [Maiorov, 1977]. Our studies of DeP supplement the plot of the relationship between synaptic transmission efficiency and synapse use/non-use (Fig. 6): the intersection of the axes (the "null" point) corresponds to the stable state (the baseline response level during rare test stimulation/"increased synaptic efficiency at the average normal level of presynaptic activity," as per Maiorov); increased synaptic activity relative to the "null" point leads initially to a decrease in efficiency (the LTD area) and then an increase (the LTP area); the ascending curve from the "null" point (the DeP area) is the mirror image of the curve of the relationship between DeP magnitude and the duration of deprivation [Popov and Markevich, 2001]. It can be suggested that the stable state (the null values on the plot) in natural or experimental conditions is determined not by the exact value of the level of afferentation (stimulation frequency) but by some range of values, which may be species-specific and dependent on the state of the animal or slice, and that the second intersection of the curve with the abscissa reflects some kind of qualitative change in the nature of the afferentation, such as a transfer from single spikes to volleys.

Conclusions

1. The development of deprivation potentiation (DeP) is mediated by independent pre- and postsynaptic mechanisms.

2. The initial peak of DeP reflects the sum of short-term presynaptic facilitation and long-term postsynaptic potentiation, with a ratio of about 1:2.

3. The development of the long-term phase of DeP and the long-term phase of LTP, associated with protein phosphorylation is due to related mechanisms which have a competitive relationship.

4. NMDA receptors are not involved in the induction of DeP.

5. The mechanism of induction of the postsynaptic component of DeP involves purine P2 receptors.

6. DeP is part of a single mechanism of plasticity associated with changes in presynaptic activity.

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