

Cloning, expression and modulation of a mouse NMDA receptor subunit

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The primary structure and presence of two forms of the mouse *N*-methyl-D-aspartate (NMDA) receptor channel subunit $\zeta 1$ have been disclosed by cloning and sequencing the cDNAs. The $\zeta 1$ subunit shows ~20% amino acid sequence identities with the rodent α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)- or kainate-selective GluR subunits and has structural features common to neurotransmitter-gated ion channels. Functional homomeric $\zeta 1$ channels expressed in *Xenopus* oocytes by injection of the subunit-specific mRNA exhibit current responses characteristic for the NMDA receptor channel such as activation by glycine, Ca^{2+} permeability, blocking by Mg^{2+} and activation by polyamine. It has been found that the $\zeta 1$ channel activity is positively modulated by treatment with 12-*O*-tetradecanoylphorbol 13-acetate (TPA).

Glutamate receptor; Glycine; *N*-methyl-D-aspartate receptor channel; Mg^{2+} block; 12-*O*-Tetradecanoylphorbol 13-acetate

1. INTRODUCTION

Glutamate receptor (GluR) channels mediate most of the fast excitatory synaptic transmission in the central nervous system and are classified into three major subtypes, that is, receptors for kainate, AMPA and NMDA based on the pharmacological and electrophysiological properties [1,2]. The NMDA receptor channel highly permeable to Ca^{2+} plays a key role in synaptic plasticity, thought to underlie memory and learning as well as development of the nervous system [3,4]. Furthermore, abnormal activation of NMDA receptor channels has been suggested to lead to neuronal cell death observed in various acute and chronic disorders such as ischemia, stroke, Alzheimer's dementia and Huntington's disease [5,6].

A family of rodent GluR channel subunits has been characterized by cloning and expression of the cDNAs [7–17]. The rat GluR1-GluR4 (or GluRA-GluRD) subunits [7–9,11] and mouse $\alpha 1$ (GluR1) and $\alpha 2$ (GluR2) subunits [10] are highly homologous with each other (~70% amino acid sequence identities), thus classified as the α subfamily of the GluR channel. The members of the α subfamily form homomeric and heteromeric channels responsive to L-glutamate, quisqualate, AMPA and kainate. The apparent affinities of these channels are higher for quisqualate and AMPA than for

kainate, indicating that the α subfamily represents the AMPA-selective GluR channels. Additional members of the GluR channel family have been identified by screening under low stringency conditions using cDNAs of the α subfamily subunits as probes. The rat GluR5 and GluR6 subunits [12,14] and the mouse $\beta 2$ (GluR6) subunit [16] are highly homologous with each other and are classified as the β subfamily of the GluR channels. The $\beta 2$ (GluR6) subunit forms homomeric channels responsive to L-glutamate and kainate, but not to AMPA, indicating that the β subfamily represents the kainate-selective GluR channel. The other subfamily γ includes the rat KA-1 subunit [13] and the mouse $\gamma 2$ subunit [17]. The KA-1 subunit expressed in mammalian cells exhibits high affinity binding for kainate. The $\gamma 2$ subunit when expressed together with the $\beta 2$ subunit yields functional GluR channels selective for kainate. Thus the γ subfamily also represents the kainate-selective GluR channels.

Further screening under low stringency conditions using these GluR subunit cDNAs as probes has revealed the presence of additional GluR channel subfamilies. Here, we report that one of these subunits, designated as $\zeta 1$, is found in two forms and constitutes functional GluR channels with characteristics of the NMDA receptor channel: the larger form corresponds to the rat NMDA receptor subunit NMDAR1 [15]. We have found that TPA treatment positively modulates the $\zeta 1$ NMDA receptor channel activity.

2. MATERIALS AND METHODS

2.1. Cloning and sequencing of cDNAs

Screening of mouse forebrain and cerebellar cDNA libraries constructed in λ gt10 under low stringency conditions with mouse $\alpha 1$ and

Abbreviations: AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; APV, D-2-amino-5-phosphonovaleate; GluR, glutamate receptor; nAChR, nicotinic acetylcholine receptor; NMDA, *N*-methyl-D-aspartate; TPA, 12-*O*-tetradecanoylphorbol 13-acetate

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$\alpha 2$ subunit cDNAs [10] as probes yielded several cDNA clones encoding a novel GluR subunit [16,17]. Additional cDNA clones encoding a novel putative GluR subunit were obtained by series of screening of the same libraries with newly isolated cDNAs as probes. The cDNA insert of some of these recombinant phage carrying $\zeta 1$ subunit cDNAs were subcloned into the *EcoRI* site of the plasmid pBluescript II SK (+) (Stratagene) to yield the plasmids pHe9, pHe12, pHeF3, pHeF32, pHeF35, pFN4 and pFN12, respectively.

The nucleotide sequence (residues -149 to 3183) of the full-length cDNA clone He12 was determined on both strands by the dideoxy chain termination method [18]. Nucleotide sequence analysis revealed that the clone HeF3 carried parts of the He12 sequence corresponding to residues -28 to 2534 and 2646 to 3183 in succession, and the clone He9 these corresponding to residues 962 to 2534 and 2646 to 2813, indicating that 111 nucleotides (residues 2535 to 2645) were missing in these clones. We conclude that the HeF3 cDNA encodes a smaller form of the $\zeta 1$ subunit, designated as $\zeta 1-2$. Partial nucleotide sequence analysis showed that the 111 nucleotide segment was present in the clones HeF35, FN4 and FN12, and was absent in the clone HeF32. Analysis of nucleotide and amino acid sequences was carried out using GENETYX software (SDC) and Gene Works (IntelliGenetics).

2.2. Expression of cDNAs

Oligodeoxynucleotide 5'-CCAGGGTGCA-3'
3'-ACGTGGT₆CC-5'

was inserted into the *Pst*I site of the pBluescript II SK (+) (Stratagene) in the same orientation as the T3 promoter to yield the expression vector pBKSA. The 3.4 kb *EcoRI* fragment from He12 was subcloned into the *EcoRI* site of pBKSA in the same orientation as the T3 promoter to yield the plasmid pBKSA $\zeta 1$. The 1.4 kb *XhoI* fragment from pBKSA $\zeta 1$, the 1.1 kb *XhoI-EcoRI* fragment from pHe9 and 3.0 kb *XhoI-EcoRI* fragment from pBKSA $\zeta 1-2$ were ligated to yield the plasmid pBKSA $\zeta 1-2$, carrying the coding sequence of the $\zeta 1-2$ subunit. The $\zeta 1$ subunit-specific and $\zeta 1-2$ subunit-specific mRNAs were synthesized in vitro with T3 RNA polymerase (BRL) using *Not*I-cleaved pBKSA $\zeta 1$ and pBKSA $\zeta 1-2$ as templates, respectively. Transcription was primed with cap dinucleotide mGpppG (0.5 mM). *Xenopus laevis* oocytes were injected with the $\zeta 1$ subunit-specific or the $\zeta 1-2$ subunit-specific mRNA; the concentration of the mRNA was 1.0 μ g/ μ l and the average volume injected was $\sim$ 50 nl per oocyte. Whole-cell currents were recorded at -70 mV membrane potential as described [10,19]. For TPA treatment, oocytes were perfused with normal Ringer's solution containing 1 μ M TPA for 10 min.

3. RESULTS

3.1. Two forms of the mouse NMDA receptor subunit $\zeta 1$

A number of cDNAs encoding a putative GluR subunit have been isolated by series of screening under low stringency conditions of mouse forebrain and cerebellar cDNA libraries using mouse GluR $\alpha 1$ and $\alpha 2$ subunit cDNAs [10] and newly isolated putative GluR subunit cDNAs [16,17] as probes. Fig. 1 shows the nucleotide sequence and deduced amino acid sequence of one of these subunits, designated as the $\zeta 1$ subunit. The initial

methionine is assigned to the first methionine residue found in a large open reading frame. The amino-terminal hydrophobic segment is assumed to represent a signal peptide as proposed for the GluR $\alpha 1$ and $\alpha 2$ subunits [10]. The proposed mature $\zeta 1$ subunit is composed of 920 amino acids with a calculated molecular weight of 103,477. The mouse $\zeta 1$ subunit shares 99.8% amino acid sequence identity with the rat NMDA receptor subunit NMDAR1 [15] and $\sim$ 20% identities with the subunits of the AMPA- or kainate-selective GluR channel [7, 14, 16, 17], thus representing the mouse counterpart of the rat NMDAR1 subunit. Analysis of several $\zeta 1$ subunit cDNA clones suggests the presence of a smaller form of the $\zeta 1$ subunit, designated as $\zeta 1-2$, whose sequence is identical to that of the $\zeta 1$ subunit except that 37 amino acids (residues 846-882) are deleted. Thus, the mature $\zeta 1-2$ subunit is composed of 883 amino acids with a calculated molecular weight of 99,313.

3.2. Structural characteristics

Analysis and comparison of the local hydropathicity profile of the $\zeta 1$ subunit suggest the presence of four putative transmembrane segments (M1-M4) in the carboxyl-terminal half of the molecule (Fig. 2). In accord with this model is the recent finding that the arginine 586 in the M2 segment of the $\alpha 2$ subunit determines the Ca^{2+} permeability of AMPA-selective GluR channels [19,20]. The $\zeta 1$ subunit contains an asparagine residue at the corresponding position in segment M2 (amino acid residue 598). In addition to the putative transmembrane regions, the region preceding segment M1 is also well conserved among GluR subunits, where the agonist binding site has been postulated [10,11]. A point mutation introduced into this region of the $\alpha 1$ subunit (mutation $\alpha 1$ -K445E) strongly reduces the EC_{50} value for L-glutamate [19]. The putative cytoplasmic domain between segments M3 and M4 contains potential phosphorylation sites for Ca^{2+} -calmodulin-dependent protein kinase type II [21] and protein kinase C [22]. Ten out of twelve potential N-glycosylation sites are found in the proposed extracellular domain.

3.3. Functional expression

The $\zeta 1$ and $\zeta 1-2$ subunits were expressed in *Xenopus* oocytes by injection of the respective mRNAs synthesized in vitro from cloned cDNAs. Oocytes injected with the $\zeta 1$ subunit-specific mRNA showed small but clear responses to 10 μ M L-glutamate, 100 μ M L-

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Fig. 1. Nucleotide sequence of cDNA encoding the mouse NMDA receptor $\zeta 1$ subunit and the deduced amino acid sequence. Nucleotide residues are numbered in the 5' to 3'-direction, beginning with the first residue of the codon specifying the amino-terminal residue of the mature subunit and the preceding residues are indicated by negative numbers. Amino acid residues are numbered beginning with the amino-terminal residue of the mature subunit and the preceding residues are indicated by negative numbers. Numbers of the nucleotide and amino acid residues at the right-hand end of the individual lines are given. The 111 nucleotides (residues 2535 to 2645) missing in the $\zeta 1-2$ subunit are underlined. The nucleotide sequence data will appear in the DDBJ, EMBL and GeneBank Nucleotide Sequence Database under the accession number D110028.

- 11		
$\alpha 1$	MPYIFAFFCTQFLQAVVQAN--FPNNIQIGQLFPNQSQEHAARFALSQ---LTEPP-K-LLPOIDIVNISDSFEM	52
$\alpha 2$	MQKINHISVLLSPVLWQLIFQVS---SNSIQIGQLFPNQSQEHAARFALSQ---FSTSEFR-LTPHIDNLEANSFAV	52
$\zeta 1$	MSTMHLTTFALLFSCSFARAACDPKIVNIQAVLSTRK--HEQMFREAVNQANKRHQSW-KIQLNATSVTHKPNAIQM	56
$\alpha 1$	TYRFCSQ-FSKQVYAF-----GFYERRIVNMLTSFGALHVCFIIT--PSFPVDTSNQFVLO-LRP-ELQEAL-ISI	119
$\alpha 2$	TNAFCSQ-FBRQVYAF-----GFYDKKSYNTITSECGTLHVSEFIIT--PSFPTDQTHPFVIO-MRP-DLQAL-LSL	119
$\zeta 1$	ALSVCEDLISQVYAILVSNPPTPNDFHTPTPVSYTAQFYRIPVLQLTIRMSIYSDKSIHLSFLRTVPPYSHQSSVWFEM	138
$\alpha 1$	IDHWKQTFVYIVDAQRQLSVLQRLVDT-AAEKWQVTAVN---ILTTTEQYRMLFQDLEKKERLVVVOCESERLNA	183
$\alpha 2$	IEYVQDNFAYLYDSRQLSTLQAVLDS-AAEKWQVTAIVQNIINNDKKDETYRSLFQDLEKKERRVILDCERDKVND	187
$\zeta 1$	MRVYNHNNHILLVSDDEGRRAQKRLLETLEERESKAENVL---QFDPQTKNVTALLMEARDLEAVIILSASEDDAAT	212
$\alpha 1$	ILQQIVKLEKNGIQVHYILANLQFMDILNKFKNESGANVIGFQLVNYDTIPARINQQWRTSDARDHTRYDWRKPHYTSA	273
$\alpha 2$	IVDQVITIGKHYKQVHYIILANLQFDDQLLKIQFGQANVSQFQIVVYDDSLASKFIERWSTLEQKVPQANTATIKVISA	277
$\zeta 1$	VYRAAAMLNMTOSQVHWLVQE--REISQNALRYAPDQIIGLQILIN-QHNESEHISDAVGQVVAQAVHELLE-KENITDPP	287
$\alpha 1$	LYDQVQVMAEAFQLRRQRIDIS-RRQNAQDCLANPAVPWQQQIDIQRALQQVFE--DLTQNVQFNEKQRRINVTILHV	350
$\alpha 2$	LYDAVQVMTAEFRNLKQRIDIS-RRQNAQDCLANPAVPWQQQVEIERALQVQVE--DLSONIKFDQNGKRIINVTINI	354
$\zeta 1$	RQCVQNTNIWHTQPLFKRVLMSKYADQVTRVEFNEQDQKFAVYSIMNLQNRKLQVQIYNQTHVIPDRNIIVPQQE	367
$\alpha 1$	IEKKHD--QIRKIQVNHEDDKFVPAATDAQAQDSSVQNRIVITITL-EDPVVHLKKNANQFEQNDRVQGVVCLAA	428
$\alpha 2$	MEIKTN--QPRKIQVWSEVDKMYVITITELPSQNDTSQLENNIVVITITL-ESPVVMMKKNHMLEQNERVEQNCVCLAA	430
$\zeta 1$	TEKPROVQMTSLKIVTIHQEPFVYVKPTMSDQTCKEEFTVNGDPMKKVICTOPNDTSPOSPRHTVPQCCV-DFCVDAI	446
$\alpha 1$	EIAKHVQVSYLEIVSDQKYGARDP---DTKANNQMYGELYVGRADVAVAPLTITLVREEVDFSKPFMSLQISIMIKK	502
$\alpha 2$	EIAKHCGFMYKLTIVDDQKYGARDA---DTKANNQMYGELYVGRADVAIAPLTITLVREEVDFSKPFMSLQISIMIKK	508
$\zeta 1$	KLARTNMFYIEVHLVADQKFGTQERVNNSNKKHNNQNGDELLSQADMIAPLTINHERAQVIEFSKPFMYQQLTILVKK	526
$\alpha 1$	PQKSKPGVFSFLDPLAYEIMCIVFAYIQVSVVFLVSRFSPYENHSEEFEEQRDQDTSQSQSNEFGIENSLMFSLQAFNQ	582
$\alpha 2$	PQKSKPGVFSFLDPLAYEIMCIVFAYIQVSVVFLVSRFSPYENHTEEFEEGRQTSQSQSSESTNEFGIENSLMFSLQAFNR	586
$\zeta 1$	-EIPRSTLDSFMQPFQSTLMLLVGLSVHVVAVMLVLLDRFSDF---GRF---KVNSEEEEDALTSSAMWFSQVLLN	598
M 1 M 2		
$\alpha 1$	QG-CDISPRSLSGRIYGVVWFITLIIISYTNLAFLTVRMVSPHIESAED--LANKTE-IAYDTLEAGSTKEFFRRS	658
$\alpha 2$	QG-CDISPRSLSGRIYGVVWFITLIIISYTNLAFLTVRMVSPHIESAED--LANKTE-IANGHLDSGSTKEFFRRS	662
$\zeta 1$	SGIQEGAPRSFSARILQNVWAGFIAMIVASYTNLAFLVLDPEERTTGINDPRLRNVSQKFIATVYKQSSVDIVFRH	676
M 3		
$\alpha 1$	KIAVFEKMMTYMKSAPSVFVRTTEGMIRVRMSKQKVAYLLEISTMNEYIEQRNPGDINMKVGNLDSKNGIATPKGISAL	738
$\alpha 2$	KIAVFDKMMTYMKSAPSVFVRTTAEQVARVRMSKQKVAYLLEISTMNEYIEQRNPGDINMKVGNLDSKNGIATPKGISAL	742
$\zeta 1$	QVE-LSTK--V-RHMEKHNV-ESAAEIAQAVRDNI-LHAFIWDIAVLEF-EASQKCDLVTTRELFERSQFGIGMRNDSW	750
$\alpha 1$	RGPNRLAVLNISELGYNINIKKMM-WYDQGECSGSGSGSKDKISALSLSNVAGVFYILVGGGLGLAMLVALLIEFCYKRAE	817
$\alpha 2$	GNAVNLAVLKLNEGGLLDKLNKKM-WYDQGECSGSGSGSKDKISALSLSNVAGVFYILVGGGLGLAMLVALLIEFCYKRAE	821
$\zeta 1$	KONVSLSLKSHENGFMEDLQKTNVRNQ--ECDSRSNAP---ATLTFFENWAGVFMLVAGGVAGIFLIFIEHAYKRHKD	824
M 4		
$\alpha 1$	SKRNKGFCLIPQQSINEAIRYSTIPRNSGAGASQSGSGSGENGRVVSQDFPKSNQSIPCMSSHSGMPLGATGL	889
$\alpha 2$	AKRMKVAKNAQNIIPSSQNSQNFATYKEGVNVVGIESVKI	862
$\zeta 1$	ARRKQMQLAFAAVNVVRKNLQDRKSGRAEPDPKKKATFRAITSTLASSFKRRRSSKDTSTGGGRGALQNKQDITVLRRAI	904
$\zeta 1$	EREQGQLQLCSRHRES	920

Fig. 2. Alignment of the deduced amino acid sequences of the mouse GluR $\alpha 1$, $\alpha 2$ and $\zeta 1$ subunits. Numbers of the amino acid residues at the right-hand end of the individual lines are given. Sets of identical amino acid residues in the homologous region are enclosed. The 37 amino acid residues (residues 846–882) missing in the $\zeta 1$ -2 subunit are underlined. The putative transmembrane segments (M1–M4) are indicated. 7 asparagine residues as potential *N*-glycosylation sites in the predicted extracellular domain are marked below with asterisks. Consensus phosphorylation sites for Ca^{2+} -calmodulin dependent protein kinase type II in the predicted intracellular domain are marked with open circles. The position of the point mutations $\alpha 1$ -K445E [19] and $\alpha 2$ -R586Q [19,20,37] affecting the agonist binding and channel properties, respectively, are indicated by arrowheads.

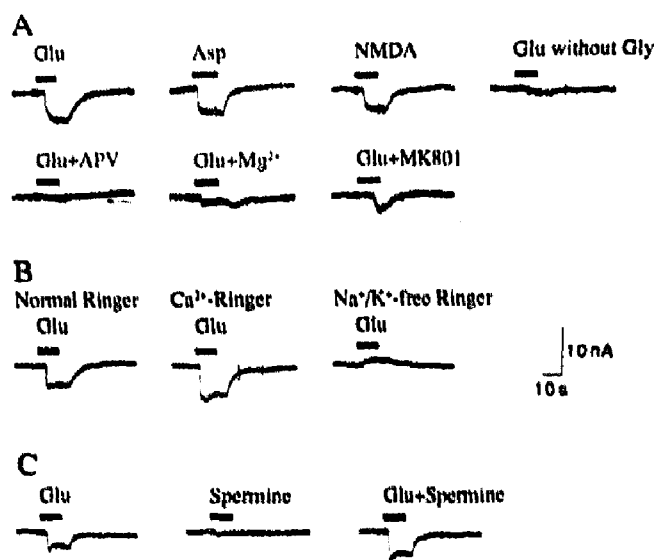


Fig. 3. Functional properties of the $\zeta 1$ subunit expressed in *Xenopus* oocytes. Agonist solutions contained 6 μ M glycine unless otherwise indicated. Membrane potential, -70 mV. Inward current is downward. The duration of agonist application is indicated by bars without taking into account the dead-space time in the perfusion system (~ 2 s). (A) Whole-cell current activated by bath application of 10 μ M L-glutamate, 100 μ M L-aspartate, 100 μ M NMDA, 10 μ M L-glutamate without glycine, 10 μ M L-glutamate with 100 μ M APV, 10 μ M L-glutamate with 100 μ M Mg^{2+} , and 10 μ M L-glutamate with 1 μ M (+)-MK801. (B) Whole-cell current responses to 10 μ M L-glutamate recorded from an oocyte in normal frog Ringer's solution (left), Ca^{2+} -Ringer's solution (middle) and Na^{+} - and K^{+} -free Ringer's solution (right). (C) Whole-cell currents activated by bath application of 10 μ M L-glutamate (left), 250 μ M spermine (middle) and 10 μ M L-glutamate with 250 μ M spermine (right).

aspartate, 100 μ M NMDA (Fig. 3A) and 100 μ M quisqualate (not shown) in the presence of 6 μ M glycine at -70 mV membrane potential. No response was detected for 100 μ M kainate and 100 μ M AMPA even in the presence of glycine. The response to 10 μ M L-glutamate was hardly detectable in the absence of glycine, an absolutely required activator of the NMDA receptor [23,24], and was diminished in the presence of 100 μ M D-2-amino-5-phosphonovaleate (APV), a specific competitive antagonist of the NMDA receptor [25] (Fig. 3A). 100 μ M Mg^{2+} , known to block the NMDA receptor channel in a voltage-dependent manner [26,27], and 1 μ M (+)-MK-801, a channel blocker of the NMDA receptor [28], reduced the response to L-glutamate (Fig. 3A). The $\zeta 1$ channel activity was attenuated by the presence of 100 μ M Zn^{2+} , reported to induce a non-competitive inhibition of the NMDA response in voltage-independent manner [29,30] (not shown). Similar results were obtained for the $\zeta 1$ -2 subunit. The homomeric $\zeta 1$ channels exhibited a clear inward current in Na^{+} - and K^{+} -free Ringer's solution containing 20 mM Ca^{2+} (Ca^{2+} -Ringer's solution [19]), whereas a marginal outward current was observed in control Na^{+} - and K^{+} -free Ringer's solution (Fig. 3B).

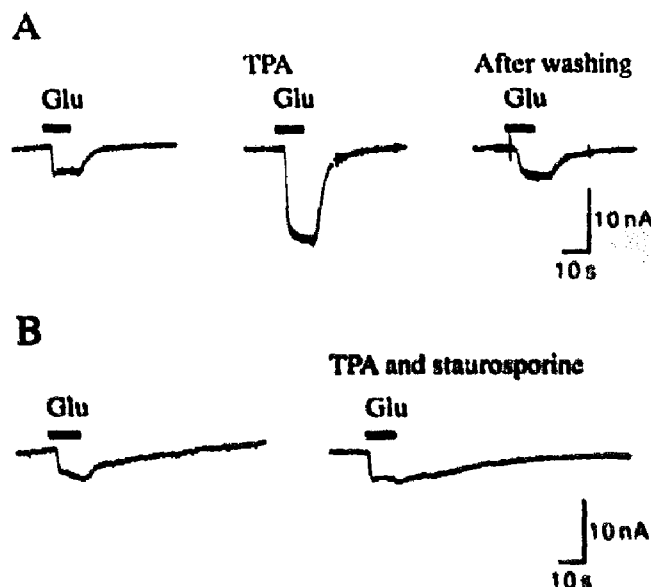


Fig. 4. Augmentation of $\zeta 1$ channel activity by TPA treatment. The experimental conditions were as in Fig. 3 unless otherwise specified. (A) Whole-cell currents activated by bath application of 10 μ M L-glutamate before (left) and after treatment with 1 μ M TPA for 10 min (middle), and after perfusion with normal frog Ringer's solution for a further 20 min (right). (B) Whole-cell currents activated by bath application of 10 μ M L-glutamate before (left) and after treatment with 1 μ M TPA in the presence of 5 μ M staurosporine for 10 min (right).

indicating that the $\zeta 1$ channels are permeable to Ca^{2+} . Spermine, reported to potentiate the NMDA receptor [31], enhanced the response of $\zeta 1$ homomeric channels by 1.4 ± 0.1 fold ($n=6$) (Fig. 3C). This effect was reversible. These pharmacological and electrophysiological properties are in good agreement with those of the NMDA-type GluR channel [1,2,31].

3.4. Modulation of channel activity

Treatment of the oocytes with 1 μ M TPA for 10 min enhanced the response of the $\zeta 1$ channels to 10 μ M L-glutamate by 2.7 ± 0.4 fold (mean $\pm$ SE, $n=10$) (Fig. 4A). The channel activity decreased gradually to the initial level by washing. This enhancing effect cannot be non-specific because the TPA treatment exerted no appreciable effect on the current amplitude of the heteromeric $\alpha 1/\alpha 2$ GluR channels expressed in oocytes (not shown). Furthermore, the effect of TPA was abolished by the simultaneous treatment with 5 μ M staurosporine, a protein kinase inhibitor [32] (Fig. 4B). Enhancing effect of spermine was observed even after treatment with TPA. The $\zeta 1$ -2 channels were similarly modulated by TPA treatment and polyamine.

4. DISCUSSION

The present investigation has shown the presence of two forms of a mouse NMDA receptor subunits, de-

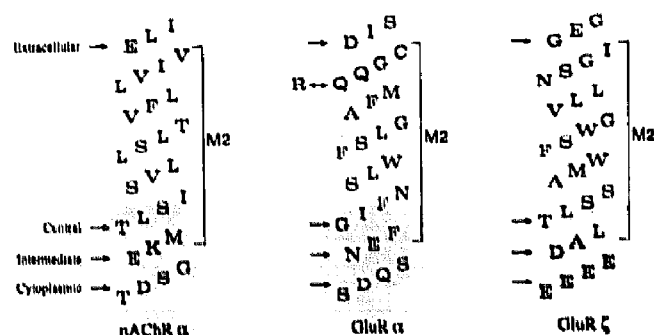


Fig. 5. Putative channel-forming regions of the GluR $\alpha 1$ and $\zeta 1$ subunits proposed by analogy of the nAChR α subunit. The one-letter amino acid notation is used. Arrows indicate the amino acid residues of the *Torpedo californica* nAChR α subunit constituting four rings as major determinants of ion flow through the channel [33,34] and the corresponding residues of the mouse GluR $\alpha 1$ and $\zeta 1$ subunits. The glutamine residue of the GluR α subunit corresponding to the arginine residue of the GluR $\alpha 2$ subunit as a critical determinant of the Ca^{2+} permeability [19,20] is also indicated.

signated as $\zeta 1$ and $\zeta 1-2$. The NMDA receptor subunit $\zeta 1$ as well as its smaller form $\zeta 1-2$ is endowed with most of the properties characteristic for the NMDA receptor, such as activation by glycine, Cu^{2+} permeability, blocking by Mg^{2+} , inhibition by Zn^{2+} and activation by polyamine [1,2,31], in accord with the rat counterpart of the $\zeta 1$ subunit [15]. In the absence of glycine, L-glutamate or NMDA alone failed to evoke the response of the $\zeta 1$ or $\zeta 1-2$ channels, in consistent with the absolute requirement of glycine for activation of rat NMDA receptor channels expressed in *Xenopus* oocytes by injection of rat brain poly(A)⁺ RNA [24]. On the other hand, the rat counterpart of the $\zeta 1$ subunit has been reported to be activated by MNDA in the absence of glycine [15], although possible contamination of glycine in the assay system used can not be excluded. The lower channel activities of homomeric $\zeta 1$ or $\zeta 1-2$ channels as compared with NMDA channel activities observed in oocytes injected with mouse brain poly(A)⁺ RNA may imply the requirement of additional subunits for the NMDA receptor channel.

Since the GluR channels of NMDA-type and non-NMDA type are structurally similar but are functionally distinct, it is of interest to precisely inspect the primary structures of the glutamate-gated ion channels. Under the assumption that the basic structure is similar between the GluR channel and the nicotinic acetylcholine receptor (nAChR) channel, one can figure out the putative channel-forming region of the GluR channels (Fig. 5). Negatively-charged and polar residues are found in the $\zeta 1$ subunit at the positions corresponding to the four rings of the nAChR channel, major determinants of the ion flow through the channel [33,34]. Asparagine residue occupies the position corresponding to glutamine or arginine of the GluR

$\alpha 1$ and $\alpha 2$ subunits, which determines the Ca^{2+} permeability of the GluR channel [19,20]. Unique to the $\zeta 1$ subunit is the presence of a contiguous stretch of negatively-charged aspartate and glutamate residues at the amino-terminal side of the segment M2. The corresponding region of the nAChR includes the intermediate ring constituting the narrowest constriction of the channel. In view of the fact that a point mutation introduced into the intermediate ring of the nAChR channel decreases not only the conductance but also the sensitivity to Mg^{2+} [33], the stretch of negatively charged aspartate and glutamate residues of the GluR $\zeta 1$ subunit may be responsible for the voltage-dependent channel block by Mg^{2+} . On the other hand, the carboxyl-terminal region of segment M3 is highly conserved among GluR subunits and thus may play an important role common to the GluR channel family.

Our finding that the $\zeta 1$ channel activity is enhanced by TPA treatment raises an intriguing possibility that the NMDA receptor channel can be regulated by protein kinases and such modulation might play a role in long-term potentiation, excitotoxicity and developmental synaptic plasticity, in which NMDA receptors are thought to mediate key steps [1-4]. In this respect, it is to be noted that potentiation of the NMDA receptor by a μ opioid has been reported to be mediated by protein kinase C [35]. Furthermore, protein kinase C has been suggested to mediate the induction and maintenance of long-term potentiation [36].

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