MINIREVIEW

Developmental Changes of Transmitter Gated Channels

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During synapse formation many developmental events are concerted to occur precisely in space and time. Some of them proceed independently from other events and some are directly induced by preceding events. Still others could occur independently but are normally influenced by others. These events are intricately interwoven to achieve a final goal, in this case, the formation of synapse. Obviously, the channels change their properties in the context of other developmental events and the topic of this review should not be considered isolated from others. But, within the scope of this mini review series I will simply examine different types of synapse to look for common aspects of channel modification. Some events might be unique in one specific transmitter system while others might occur universally. Hopefully this sorting will highlight the obligatory alterations which take place during this period of development and shed light on the underlying mechanism.

Among various synapses in the nervous system the neuromuscular junction in skeletal muscle has been studied most extensively in the past and consequently we now know a great deal about this synapse in the adult animals. We also have abundant knowledge on various processes which occur during the formation of synapse. In vertebrates the transmitter at this synapse is acetylcholine (ACh). Because of readily available materials, such as *Torpedo* electric organ, and invaluable tools, such as an irreversible blocker, α bungarotoxin, ACh receptor channels are most extensively studied in biochemistry, physiology, biophysics, and molecular biology. By all means the vertebrate neuromuscular junction will serve as a model with which phenomena in other transmitter systems will be compared. Recently, however, cDNA clones for other types of receptor channels were isolated and now widely spread researches are being undertaken in various fields. I shall first review the ACh receptor channel in the vertebrate neuromuscular junction. Pieces of information on other types of receptor channels will be considered in relation to the similar changes in the ACh receptor channel. Similarities and differences will be pointed out. Finally common denominators will be searched and discussed.

ACh RECEPTOR CHANNELS

In 1972 at the frog neuromuscular junction Katz and Miledi [1] first demonstrated using the technique of ACh-induced voltage noise analysis that the elementary voltage deflection was greater in denervated muscle than in innervated muscle. This observation was interpreted as an increased duration of the underlying elementary conductance change following denervation. This interpretation was later supported by the noise analysis of ACh-induced membrane currents in denervated frog muscle fibers [2]. Subsequently, channels similar to those in the denervated muscle were found in embryonic myotubes and a developmental alteration from slow embryonic to fast adult type was reported [3,4]. Instead of describing chronologically through literature I will document overall developmental changes mainly in the well-studied *Xenopus* nerve-muscle system and compare those with findings in other animals. It appears that the basic changes are the same in different animals although we have more knowledge in some animals but less in others. Brief reviews on this subject have been published previously elsewhere [5-7].

Another type of ACh receptor channels, nicotinic ganglionic receptor channel in neurons, is in many ways different from ACh receptor channels on skeletal muscle. I will not review those receptor channels. But, a recent study on chick sympathetic neurons demonstrated that there are multiple types of channel events at the time of synapse formation [8].

Two types of ACh receptor channels

Normally ACh receptors appear on the surface membrane as soon as myoblasts fuse to form myotubes [9]. The receptor density at this early stage is low but those receptor channels are functional. In the *Xenopus* tadpole tail, muscle cells are mononucleate even in well-advanced stages. In those mononucleate myocytes, two types of ACh receptor channels were found at the time of earliest receptor appearance [10-12]. They will be referred to in this review as "embryonic" and "adult" type for reasons which will become obvious later. These two types of channels are functionally different in various aspects. In *Xenopus* myocytes the embryonic type channel has the mean open time of about 7 ms and the single channel conductance of about 45 pS while the adult type has the shorter mean open time of about 3 ms and the larger channel conductance of 60 pS.¹

¹ The apparent mean open time and the single channel conductance vary with experimental conditions. Among various factors the greater membrane potential (hyperpolarization) and lower temperature prolong the mean open time whereas lower temperature and higher divalent cation concentration, especially Ca^{2+} , reduce the single channel conductance. Therefore, these values are different from one report to another even from the same animal. But the relative values are similar. That is the mean open time is about twice longer in the embryonic type and the single channel conductance is about 50% smaller than the adult type.

At early stages embryonic channels are dominant and the relative proportion of the embryonic type decreases as the animal develops. Meanwhile these two types are well mixed everywhere on the muscle surface, including the postsynaptic membrane. As a consequence the decay time course of synaptic current is slower in early stages and progressively become faster as development proceeds.²

When single channel current recording was carried out, patches always contained two types of channel events although in early stages adult type channel events were quite infrequent [11]. The relative proportion of these two types of channel events is probably independent from the channel density. In other words they are well mixed everywhere on the muscle surface. In *Xenopus* myocytes the mixed population of channels are found at the extrajunctional as well as at the junctional region even at stages when receptors have been accumulated and the channel density is more than 10 times greater at the junction [15, 16].

Relative proportions of these two types of channel progressively change during developments as shown in Fig. 1. As a consequence at the adult neuromuscular junction, adult type receptor channels are exclusively found.

This switch of channel type occurs early during development when the neuromuscular junction is forming. Therefore, it is reasonable to suspect that this conversion is induced by the nerve contact. In fact, when soleus muscle in the neonatal rat was denervated before the channel open time shortened, the developmental change in the channel kinetics was blocked [17]. On the other hand, it has been shown that this change takes place in cultured *Xenopus* myocytes in the absence of nerve. The time course of the switch is exactly the same as in the animal [18–20] indicating that this developmental change is independently programmed to occur in *Xenopus* muscle cells. It is intriguing, in this case, how the timing is coordinated with other changes in nerve and muscle. This nerve-independent alteration of the channel properties, however, has not been documented in other animals.

In neonatal rat soleus muscle two types of ACh receptor channels were found [3, 21]. The mean channel open time was about 4 times longer in 8-d-old rats than in 18-d-old ones. Similar shortening of the mean open time was observed in neonatal omohyoideus muscle but in earlier times than in soleus muscle [3]. This observation indicates that the channel conversion occurs at different times in different muscles probably depending on the time when each muscle develops.

Interestingly, in the chick there is only one type of channel in terms of the apparent channel open time throughout posthatch development [4]. With single

² When a large number of homogeneous receptor channels open instantaneously due to a brief jump of the transmitter concentration in the synaptic cleft, the decay time course of the synaptic current is expected to follow an exponential. The time constant of the exponential will be equal to the mean open time. This was verified at the frog neuromuscular junction when the time course of synaptic current and the channel open time were compared [13]. When two types of channel were mixed in newborn rats two exponentials were needed to adequately fit the decay time course of the synaptic current [14].

Fig. 1. The increase in the percentage of high-conductance channel events (adult type) with successive stages of development. For each experiment, the percentage of high-conductance channel events during the recording period was determined. Each point is the mean $\pm$ SEM for the patches from the indicated developmental stages. The number of patches examined is indicated for each point. The percentage of high-conductance channel events began to increase appreciably around stage 34 (reproduced with permission from [11]).

channel recording in cultured chick myotubes, however, Auerbach and Sachs [22] found two types of events with mean unitary conductances of 35 and 60 pS but they were not different in the mean open time.

Thus in the chick it is plausible that the switch from one type to the other is occurring but with the similar mean open times it was not detected in the noise analysis carried out by Schuetze [4].

The channel conversion is not correlated with the receptor turn-over rate

The metabolic turnover rate of the receptors newly appeared in the extra-junctional region after denervation of adult muscle is much faster than that at the junction (for review see [23]). Similarly, the receptors in the embryonic muscle turn over rapidly. However, this change does not correlate with changes in channel conversion. Michler and Sakmann [24] have shown in rat soleus muscle that receptors present at day 4 after birth are already metabolically stable with a half degradation time of more than 9 d; however, channel conversion occurs between day 8 and day 18 of postnatal development, with a half conversion time of about 3 to 4 d. This is taken as evidence that, during synaptogenesis, receptor turnover and channel conversion are controlled by different mechanisms.

This point was also addressed in cultured *Xenopus* myotomal muscle cells where it was found that the fast turnover rate (a half degradation time of 52 h at room temperature) did not change over the course of the change in channel kinetics [25].

Molecular basis of two types of channels

cDNAs for four subunits, α , β , δ , and γ of the ACh receptor channel were first cloned for the extrajunctional (embryonic) type [26]. When messenger RNAs for these subunits were injected into *Xenopus* oocytes the expressed receptor channel events were exclusively of the low conductance, slow embryonic type. On the other hand when the ϵ subunit mRNA [27] substituted for the γ subunit mRNA the resulting ACh receptor channels were all of high conductance, fast adult type. The mean open time was about two times longer for the combination of mRNAs including γ subunit mRNA than the other combination.

Furthermore the γ subunit mRNA was found abundantly in bovine fetus at early stages (3–5 months of gestation) but was hardly detectable after birth while considerable amounts of ϵ subunit mRNA appeared only at postnatal stages, and it was not detectable at early fetal stages (3–4 months of gestation). At later fetal stages (7–8 months of gestation), appreciable amounts of the γ and ϵ subunit mRNAs coexisted. These findings strongly suggest that the ACh receptor channel in bovine fetal muscle is assembled from α , β , δ , and γ subunits whereas the endplate channel in adult muscle is assembled from α , β , δ , and ϵ subunits.

Since these subunits are probably assembled when they are inserted in the membrane, the developmental switch from the embryonic to adult type is likely due to replacement of the whole molecule of the former type by the latter, rather than molecular modification of the channel in the membrane. In the case of cultured *Xenopus* myocytes the switch occurs in several days [11], while the half turnover time of the receptor is 52 h [25]. Thus there is enough time for the replacement of old channels with new ones with different properties. However, in the rat, Brenner and Sakmann [28] reported that the turnover time was much longer than the time course of the switch and concluded that the switch is due to modification of the existing channel. This controversy has to be settled in future experiments.

The burst duration³ also shortens within each type of channel

In cultured *Xenopus* embryonic muscle cells Leonard *et al.* [19] first reported

³ There is often confusion in the usage of the term, the mean channel open time. In early days it was understandably called the mean open time. Then, brief closures within an open episode were found as the time resolution of the recording system improved [29]. It became, then, more appropriate to call an open episode a burst and to measure the burst duration since the brief closures within a burst are usually close to the limit of recording system and many closures are not detected. This is also reasonable considering the synaptic function because these brief closures do not significantly contribute to change the membrane potential. The terms, mean open time, apparent mean open time, and mean burst duration, can be deemed as the same in this context.

that the mean burst duration of the embryonic type channel was long and progressively shortened as muscle cells developed. This observation was further investigated by Rohrbough and Kidokoro [11] in the same preparation. As shown in Fig. 2 the mean burst duration of the embryonic type channel was 20–25 ms at stages 20 to 25 and progressively decreased within one and a half days to about 6 ms at the stage 45 (open circles).

This change in the embryonic type channel open time is also observed *in vivo*, Owens and Kullberg [20] described a 2- to 3-fold decrease in the mean open time of the low conductance channel between stage 19–30 and stage 33–50. They recorded ACh receptor channel activities at the extrajunctional area of myotomal muscles in the *Xenopus* tadpole. This shortening of the mean burst duration also occurs in the adult-type channel (filled circles) although the extent of change is less marked.

Fig. 2. The decrease in burst duration with development. Low-conductance channels (embryonic type) are shown with open circles and high-conductance channels (adult type) with filled circles, all burst durations were measured at 50 mV applied potential. Each point is the mean burst duration ($\pm$ SEM) of the patches from the indicated stage. For muscle past stage 35/36, most patches yielded both low- and high-conductance channel burst durations. Each point shown represents one stage (e.g. stage 30) or a two-stage group (e.g. 24/25) corresponding developmental age (in hours post-fertilization [55]) for the stages studied is on the lower axis. Note that the stages are not linearly related to time. The ACh concentration was 150 nM in most cases (a few experiments were done at 100 or 200 nM) (reproduced with permission from [11]).

The observed decrease in the burst duration is largely complete after the second day in culture. By this time, muscle cells have a mixed population of old and young ACh receptors present in the membrane due to the metabolic turnover of receptors. Therefore, the fact that at this stage all receptor channels have short burst durations indicates that newly synthesized receptors also have similar channel properties to existing ACh receptors in the surface membrane. That is, at later stages even newly inserted channels have shorter burst durations.

It should also be noted that these changes in the burst duration occur in the absence of nerve although *in vivo* these changes occur during the period when the neuromuscular junction is forming.

The molecular basis of this alteration is not clear. It has been suggested that ACh receptor channels which are inserted into the membrane at early stages undergo post-translational or post-insertional modification to shorten the burst duration [19, 30]. The developmental decrease of the burst duration of low conductance (embryonic type) channels occurs without protein synthesis or without incorporation of new ACh receptors in the membrane [30]. Some form of modification, such as phosphorylation or glycosylation, of existing ACh receptors is plausible since the decrease in the burst duration occurs more rapidly than the rate of ACh receptor turnover.

This type of shortening of the burst duration has never been documented in any other animals. It is desirable to investigate closely the mean burst duration at very early stages during myotube formation, for example, in the rat.

GLUTAMATE RECEPTOR CHANNELS

Different types of glutamate receptor channels are mixed in the subsynaptic membrane in the mammalian central nervous system. To examine the developmental changes of channel kinetics one type of channel has to be isolated.

Hestrin [31] recorded evoked glutamatergic synaptic currents in superior colliculus slices from rats. At the holding potential of +40 mV the synaptic potential was blockable with an NMDA antagonist, APV, while that observed at -80 mV was blocked by a non-NMDA antagonist (CNQX). Thus he separated NMDA and non-NMDA component of the synaptic current and found that the decay time constant of the NMDA component shortened during the period between 10 and 33 d after gestation. He also noted that the amplitude of miniature synaptic currents of the NMDA component did not change during the same period, which suggests that the channel density was not changing. He also examined and showed in excised patches brief activation of NMDA receptors exhibited a decay time course similar to that of the NMDA component of synaptic currents. Thus he concluded that the shortening of the synaptic current decay was due to changes in channel kinetics and not due to other factors, such as, faster transmitter removal.

He further examined the property of miniature synaptic currents and com-

pared it between 13- and 17-d-old rats. The decay time constant was about 3-fold shorter in the latter but the amplitude was similar. The averaged time course of miniature synaptic currents was similar to that of evoked synaptic currents. These changes in the time course of synaptic currents are similar to what was observed at vertebrate neuromuscular junctions. However, it is not clear whether these changes are due to a switch of channel types or due to shortening of the mean open time within one type of channel.

He did not examine the changes in non-NMDA receptor channels.

Recently, Carmignoto and Vicini [32] reported essentially the same findings as Hestrin [31] in the rat visual cortex. Excitatory postsynaptic currents (e.p.s.c.'s) mediated by NMDA receptors were isolated by pharmacological means using non-NMDA antagonist (NBQX). The decay phase of e.p.s.c.'s was well fitted with one exponential or two exponentials. In the rats younger than 15 d after gestation the percentage of the slow component was predominant, more than 90%, whereas in rats older than 30 d it decreased to about 20%. The time constants of each component did not change during this period nor did the amplitude and rise time. The same results were found in miniature e.p.s.c.'s. They also examined unitary NMDA channel currents in excised outside-out patches. Brief application of L-glutamate caused prolonged channel activities in 12- to 14-d-old rats whereas it shortened drastically in 30- to 35-d-old rats. Thus they concluded that the developmental change of the e.p.s.c. decay time is due to alteration of channel kinetics.

They also pointed out that the slow component of NMDA-type e.p.s.c.'s was significantly larger in rats reared in the dark than in rats reared in the light. This observation is consistent with their hypothesis that the developmental decrease of the NMDA-type e.p.s.c. duration plays a crucial role in the synaptic plasticity of the developing visual cortex. This is in contrast to the finding at the neuromuscular junction where the channel conversion is not correlated with nerve activities [16].

The transmitter at the neuromuscular junction of crustaceans and insects is glutamate [33, 34]. The properties of these glutamate receptor channels are different from any of the glutamate receptor channels found in the mammalian brain. A cDNA for the *Drosophila* glutamate receptor channel subunit has been cloned which has homology of only 30% to any of those types in the mammalian CNS [35].

Recently, the kinetics of the glutamate receptor channel were studied in myotubes cultured from *Drosophila* embryos [36]. There was only one type of channel in terms of unitary conductance. Kinetically also it appeared that there is only one type of channel in culture and the mean burst duration did not change although they examined cultures up to about 2 weeks old. It is plausible that the developmental change which would have occurred in the animal does not take place in culture.

Various types of glutamate receptor channels are cloned including NMDA and non-NMDA types. Each type of them has several subunits. Combination of these

subunits will produce a large variety of channels with slightly different properties. The developmental changes of the channel kinetics are likely, at least partly, due to substitution of one or more of subunits. Various subunits in NMDA receptor channels do change their distribution in the mouse brain as demonstrated by *in situ* hybridization using mRNA specific to each subunit [37]. It is interesting to know what kinds of subunits are expressed in superior colliculus during 13 to 30 d after gestation when the kinetic change was demonstrated [31], or in visual cortex [32].

GLYCINE RECEPTOR CHANNELS

The glycine receptors are mainly distributed in the spinal cord and brain stem of adult animals and are coupled to Cl^- channels. Thus, activation of the receptor channel exerts inhibitory effects on neurons by causing membrane hyperpolarization. Strychnine is a specific antagonist.

Four subunits are known, namely, $\alpha 1$, $\alpha 2(\alpha 2^*)$, $\alpha 3$, and β [38].

Developmental alterations of the glycine receptor channel were observed in the following experiments [39,40]. An mRNA isolated from the rat spinal cord was injected into *Xenopus* oocytes and glycine-responsive currents thus expressed were measured. The apparent molecular weight of mRNAs isolated in this manner from embryos and neonates was smaller than those from adults [39]. Antisense DNA to $\alpha 1$ subunit blocks expression of mRNA from adult animals but not that from young animals when injected into *Xenopus* oocytes [41]. Furthermore antigenicity of the glycine receptor protein differs between young and adult rats [40]. $\alpha 1$ mRNAs are less abundant in embryonic or neonatal rat brain. These results suggest that the $\alpha 1$ subunit is a major constituent of the adult glycine receptor channel whereas the $\alpha 2$ subunit is dominant in embryonic and neonatal animals.

Homomeric expression of $\alpha 1$ subunit mRNAs in *Xenopus* oocytes resulted in glycine-responsive channel activities with an arithmetic mean open time of 4.56 ms whereas that of $\alpha 2$ subunit mRNAs showed a prolonged open time of 272 ms. Furthermore in excised patches from rat spinal cord slices, glycine receptor channel events had the mean open time of 7.29 ms in 22-d-old rats whereas in 20-d-old embryos it was 39.9 ms. There is a clear progressive decline of the mean open time in between these two time points. In accord with this shortening of channel kinetics the decay time constant of glycinergic synaptic currents also decreased during this period. It was 27.0 ms in embryonic day 20 whereas 10.2 ms in postnatal day 22. In between these two time points there is a progressive decrease in the decay time constant (Fig. 3) [42].

All these findings are consistent with the idea that the embryonic-type glycine receptor channel has the $\alpha 2$ subunit and that in the adult animal has the $\alpha 1$ subunit. Apparently there is a switch of a subunit during development as found in the ACh receptor channel.

In *in situ* hybridization $\alpha 2$ mRNAs are found easily in spinal cords of embryos and neonates but are not detected in adults. However, in 3-d-old neonates mRNAs

Fig. 3. Glycinergic IPSCs evoked in dorsal horn neurons. (A) Averaged IPSCs (from 17-92 records) in a spinal neuron at different developmental periods. Vertical calibration, 100 pA for E20 and P4, 140 pA for P16 and P22, and 200 pA for P8. Holding potential was 83 mV. (B) Relationship between the mean decay time constant and rise time (10-90%) of individual IPSCs recorded from an E20 (closed circles) and a P16 (open circles) neuron. (C) Developmental changes in the mean decay time constant of averaged IPSCs. Data points and bars indicate mean $\pm$ SD, derived from 11-13 neurons. The mean rise times (10-90%, $\pm$ SD) of averaged IPSCs were 0.91 ± 0.16 , 0.92 ± 0.20 , 0.86 ± 0.18 , 0.85 ± 0.20 , and 0.76 ± 0.22 ms for E20, E4, E8, E16, and P22 animals, respectively (reproduced with permission from [42]).

for the $\alpha 2$ subunit are abundantly expressed not only in the spinal cord but also in cerebral cortex and hippocampus [43]. This result is in accord with the finding that in rat hippocampal neurons glycine causes membrane potential changes in neonates but not in adults [44]. Since high affinity glycine binding sites are mostly in the spinal cord and brain stem in the adult animal [45], these results suggest that even hippocampal neurons glycine receptor channels are expressed transiently in younger animals. Whether these transiently expressed glycine receptor channels are the embryonic type remains to be clarified.

Kuhse *et al.* [46] isolated the $\alpha 2^*$ subunit from a neonatal rat brain cDNA library. The amino acid composition of the $\alpha 2^*$ subunit is very similar to that of the $\alpha 2$ subunit. Homomeric expression of the $\alpha 2^*$ mRNAs results in glycine responses which have low sensitivities to glycine as well as strychnine. Site-specific mutagenesis has revealed that an amino acid at position 167 in $\alpha 2^*$, namely, glycine, has a strong contribution to this pharmacological change. A probe specific to the $\alpha 2^*$ subunit hybridized with mRNAs isolated from newborn rat spinal cords but not from those of adult animals [46].

It is now known that glycine receptor channels formed by homomeric expression of the $\alpha 2$ subunit is sensitive to strychnine [43] and that glycinergic IPSPs in embryonic and neonatal rats are blocked by a low concentration, 500 nM, of strychnine [42]. Thus, it is not obvious how the $\alpha 2^*$ subunit is contributing to assembly of the embryonic glycine receptor channel. Furthermore the glycine receptor channels in juvenile neurons are sensitive to glycine [42] unlike the homomeric channel formed with the $\alpha 2^*$ subunit. In all, the role of the $\alpha 2^*$ subunit in the developing brain is not clear.

If the progressive shortening of the mean open time of the glycine receptor channel is due to a switch in the subunit from $\alpha 2$ to $\alpha 1$, a question arises whether during the transition period various hetero-oligomeric receptors appear or two homo-oligomeric receptor channels are mixed in a progressively changing proportion. The latter was found in the case of ACh receptor channels as described above.

GABA RECEPTOR CHANNELS

Two distinct types of GABA receptors are in the mammalian brain. The GABA_A receptor is directly coupled to a channel which is selectively permeable to Cl⁻ ions. Bicucullin or picrotoxin blocks this type of receptor and benzodiazepine enhances the GABA-activated currents. On the other hand, activation of the GABA_B receptor results in the membrane permeability change to K⁺ ions. The coupling between the receptor and the K⁺ channel is indirect via second messengers. Baclofen is a specific agonist for the GABA_B receptor. Here I shall describe findings only on the GABA_A receptor.

GABA_A receptor channels are extremely heterogeneous. Altogether 13 subunits are described and each of them changes their expression in time and space in the developing rat brain [47]. If the functional receptor channel is pentameric the

permutations of these 13 subunits are enormously large. There must be a certain number of preferred permutations which are used in the animal in a meaningful way. When different combinations of these subunits are expressed in *Xenopus* oocytes application of GABA elicited currents which have the reversal potential at the Cl^- equilibrium potential. The current-voltage curves of the GABA-induced macroscopic current are all outwardly rectifying. The sensitivities to GABA are, however, different among various combinations of subunits [48].

Sigel *et al.* [49] compared functional properties of ion channels expressed in *Xenopus* oocytes after injecting various combinations of six rat subunits. Their intention was to deduce minimal requirement of subunits in order to reproduce such channel properties as a high sensitivity and cooperativity to GABA and sensitivities to benzodiazepine, picrotoxin, and barbiturate. They found that $\alpha 5$, $\beta 2$, and $\gamma 2$ are minimally required to reproduce the above properties. However, the stoichiometry of these subunits in the normally functional receptor channel is not known.

Interestingly, in the rat up to postnatal 8 to 12 d activation of GABA_A receptor channels depolarizes neurons but subsequently the response turns into hyperpolarization as in the adult animal. This change in the polarity of the response is due to alteration of Cl^- ion distribution and not due to modification of the channel ion selectivity [50].

What is the role of this depolarizing GABA response? The GABAergic system appears earlier in the developing brain than excitatory glutamatergic connections. Therefore it is possible that the response plays a role in early formation of neuronal networks. Along this line, it has been suggested that the depolarizing response may serve as a trophic mediator by allowing Ca^{2+} ions to enter the postsynaptic cells [51].

The GABA_A receptors in neonatal neurons are also different pharmacologically. GABA currents in neonatal neurons are potentiated by barbiturates but are insensitive to benzodiazepines [52]. It is likely that subunit compositions are different in different parts of the developing brain as demonstrated with the *in situ* hybridization studies [47] and as a result the receptor channel may have different functional properties.

The channel properties at the single channel level have not yet been studied in neonatal neurons. Whereas the ion permeation mechanism of the GABA-activated Cl^- channel has been studied in detail in adult mouse spinal cord neurons [53]. In other transmitter activated channels the channel kinetics becomes faster as development proceeds. Whether the same rule prevails in the GABAergic system is yet to be determined.

DISCUSSION

Among many neurotransmitter systems in the CNS, I have reviewed only four of them in this article. One transmitter does activate more than one type of

receptor channels. For example, ACh activates nicotinic receptors in skeletal muscle, nicotinic ganglionic receptors in neurons, and muscarinic receptors. Thus, the majority of receptor channels was not examined in this review. In fact, our knowledge on other systems is fragmentary. Nevertheless, common developmental changes are emerging. Namely, the embryonic receptor channels generally have slower kinetics than the adult ones. The conversion of kinetics from slow to fast occurs as the synapse matures. Whether or not this rule is universally true has to be further investigated. The underlying mechanism for this conversion could be complex but probably, at least partly, involves subunit substitution.

The developmental changes of channel kinetics were first discovered in the ACh receptor channel in skeletal muscle and most extensively studied. There are two fundamentally different alterations which occur concurrently during the period of synapse formation. Both changes result in faster closing of the channel. It is intriguing to speculate the significance of these modifications. The decay phase of synaptic currents at the neuromuscular junction is determined by the channel kinetics. If the mean open times become shorter the synaptic currents terminate faster which will result in smaller and briefer synaptic potentials. This change is in favor of better time resolution for faster information processing. At the neuromuscular junction the movement might become faster and agile provided that cholinesterase develops in time and contractile apparatus can respond accordingly.

On the other hand embryonic-type channels open longer and thus can carry more charges per opening. This is an advantage for synaptic transmission when the receptor density is low as in early synapses. The synaptic transmission at early periods may not require good time resolution and may serve as a means for trophic interactions. We have demonstrated that twitchings caused by spontaneous release of ACh at the early synapse facilitate formation of cross-striation in cultured *Xenopus* myotomal muscle cells [54]. For alignment of myofibrils periodic contractions may work as a facilitatory factor.

These arguments based on the difference of the channel open time might not be adequate because it is known in the chicken the shortening of the channel open time does not occur [4]. Yet chicks develop normally just as any other animals.

An alternative idea was suggested by Dr. Paul Brehm some time ago (personal communication). At the neuromuscular junction it is known that after denervation, extrajunctional (embryonic) receptor channels appear anywhere on the muscle surface. This can be considered as preparatory measure for contact with incoming regenerating nerves and for quick formation of functional synapse at the earliest occasion. Obviously, for the animal who is suffering from denervating injuries a quick recovery of neuromuscular transmission is a matter of survival. It is conceivable that the embryonic type receptor channel is suited for synthesis in a large quantity in a short period of time. Therefore, during *de novo* formation and regeneration after denervation when rapid synthesis is required the embryonic type channel becomes dominant. Of course we are left with a question why the embryonic type receptor channel is easier to synthesize than the adult type.

Whatever the significance of these developmental changes, we now know that at least two other types of receptor channels, namely, NMDA type glutamate and glycine receptor channels, undergo a similar developmental process. As studies are extended to other types of receptor channels more general features will be revealed.

It appears that the transmitter activated channels are dynamically changing their properties during development. Our next task will be to elucidate how these changes are reflected in the behavior of animals. Parallel studies of channel kinetics and detailed behavioral observations of mutant animals are needed to solve this question. In doing so we might be able to correlate properties of channels in a specific circuitry with animal behaviors.

Key words: development, receptor channels, channel kinetics, synapse formation, receptor subunits.

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