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Prenatal Expressions of Hyperpolarization-activated Cyclic-Nucleotide-Gated Channel (HCN) Genes in Dysplastic Hippocampi in Rats

Sıçan Hipokampusunda Hiperpolarizasyon-aktifli Siklik-Nükleotid-Geçiş (HSN) Kanal Genlerinin Doğum Öncesi Salınımları

Cihan İŞLER[1]
Taner TANRIVERDİ[2]
Ersen KAVAK[3]
Galip Zihni SANUS[4]
Mustafa Onur ULU[5]
Gözde ERKANLI[6]
Ahmet KOMAN[7]
Necla Birgül İYİSON[8]
Mustafa UZAN[9]

1 Department of Neurosurgery, Bagcilar Training and Research Hospital, Istanbul, Turkey.
2 Department of Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada.
3,7,8 Department of Molecular Biology and Genetics, Boğaziçi University, Istanbul, Turkey
4,5,9 Department of Neurosurgery, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey.
6 Department of Histology and Embryology, Medical Faculty, Marmara University, Istanbul, Turkey.

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ABSTRACT

AIM: Hyperpolarization-activated cyclic nucleotide-gated (HCN or h-channel) channels mediate hyperpolarization-activating currents in the hippocampus and neocortex. The aim of this study is to present prenatal h-channel gene expressions (HCN1 and HCN2; HCN1-Entrez-Gene ID: 84390; HCN2-Entrez Gene ID: 114244) in dysplastic hippocampal pyramidal neurons induced by in utero irradiation in rats.

MATERIALS and METHODS: Time-pregnant Wistar albino rats were irradiated and the dysplastic hippocampus in their 2 month-old litters was studied. Gene expression was studied by RNA extraction and polymerase chain reaction methods.

RESULTS: None of the rats showed seizure activity. mRNA levels of HCN1 and HCN2 genes were decreased especially in the CA1 and CA3 pyramidal neurons in the hippocampi of experimental rats; however, the differences were not significant compared to controls. In CA2, mRNA levels of both genes were increased and this rise did not reach significant level. The CA4 sub-region showed a different pattern of expression: HCN1 increased but HCN2 decreased insignificantly compared to controls.

CONCLUSION: Our results demonstrated that dysplastic neurons showed decreased levels of mRNA expression of HCN1 and HCN2 genes, in particularly CA1 and CA3 pyramidal neurons. The rationale for how these changes contribute to epileptogenesis in dysplastic tissues still requires further studies.

KEY WORDS: Dysplasia, HCN1, HCN2, Gene expression, Hippocampal sclerosis, Temporal lobe epilepsy

ÖZ

AMAÇ: Hiperpolarizasyon-aktifli siklik nükleotid-geçiş (HSN veya h-kanal) kanalları hipokampus ve neokortekste hiperpolarizasyon akımlarını düzenlerler. Bu çalışmanın amacı doğum öncesi radyasyona maruz bırakılan sıçanlarda geliştirilen displastik hipokampus piramidal hücrelerde h-kanal gen (HCN1 and HCN2; HCN1-Entrez-Gene ID: 84390; HCN2-Entrez Gene ID: 114244) dağılımlarını ortaya koymaktır.

YÖNTEM ve GEREÇLER: Hamile Wistar albino sıçanlar radyasyona maruz bırakılmış ve displastik hipokampuslar 2 aylık yavrularda çalışılmıştır. Genler, ribonukleik asit (RNA) çıkarma (extraction) ve polimeraz zincir reaksiyon yöntemleri ile çalışılmıştır.

BULGULAR: Hiçbir sıçanda nobet görülmemiştir. HCN1 ve HCN2 mRNA seviyelerinin ozellikle CA1 ve CA3 piramidal noronlarda azaldığı tespit edilmiştir fakat kontrol gurubu ile karşılaştırmalarda anlamlı bir fark bulunamamıştır. CA2 noronlarda her iki genin mRNA seviyeleri artma göstermiştir fakat farklar anlamlı bulunamamıştır. CA4 alt bolgesi farklı salınım şekilleri göstermiştir: Kontrol gurubu ile karşılaştırıldığında HCN1 anlamlı artarken HCN2 anlamlı bir şekilde azalmıştır.

SONUÇ: Sonuçlarımız göstermiştir ki hipokampus sklerozuna bağlı gelişen mezsiyal temporal bölge epilepsilerinde önemli rol oynayan CA1 ve CA3 alt bölgelerinde HCN1 ve HCN2 mRNA genlerinin salınımları azalmaktadır. Displastik dokulardaki bu değişikliklerin epileptojeneze nasıl katkı sağladıklarının arkasında yatan gerçeklerin ortaya konması için ilave çalışmalara ihtiyaç vardır.

ANAHTAR SOZCÜKLER: Displazi, Hipokampal skleroz, HCN1, HCN2, Gene ifadesi, Temporal lob epilepsi

Correspondence address:
Taner TANRIVERDİ
Department of Neurosurgery,
Montreal Neurological Institute and
Hospital, 3801 rue University, suite 109
Montreal, Quebec H3A-2B4, Canada
Phone : (514) 398-1935
Phone : 0015142862621
Fax : (514) 398-2811
E-mail : tanerato2000@yahoo.com

INTRODUCTION

Mesial temporal lobe epilepsy (MTLE) with hippocampal sclerosis (HS) is the most common form of epilepsy syndromes and almost one-third is drug-resistant (14). Surgery on MTLE has been shown to be an effective treatment, leaving 75-85 % of patients seizure free (25,37). Accumulating evidence suggest that the entorhinal cortex layer III pyramidal neurons play an important role in either induction or maintenance of MTLE (1,2). However; whether the changes in the neuronal network functions in the mesial temporal lobe structures are either a cause to or a consequence of the ongoing seizure activity has not yet been answered. Every effort including clinical and laboratory studies made so far has tried to provide a reasonable answer with respect to the molecular basis of this cause and effect. In recent years, studies have shown that any human epilepsy syndrome associated with a gene mutation has a dysfunction in the ion channel subunits (35). Thus, it is clear that until the last 10 years, there has been little evidence that alterations of an ion channel function causes any human epilepsy syndrome. The h-channels (for Ih, the current produced by h-channels) are a group of ion channels, whose channelopathy has been implicated in epileptogenesis, although the yings and yangs or by commonly saying, the multiple personalities of the h-channels and their roles in epilepsy have been reported (9,15,22,26,28-31,33). The hyperpolarization -activated cyclic nucleotide-gated (HCN or h-channel) channels are voltage-gated ion channels that mediate hyperpolarization-activated currents (Ih) (13). The most unusual feature of h-channels is that they open as a response to hyperpolarization (hence the "h" in the h-channel) rather than depolarization, which is the main triggering factor for the action of all other voltage-gated channels. The current, Ih, produced by the h-channels, maintains the resting membrane potentials, shapes and synchronizes rhythmic activities and regulates the dendritic depolarization in the neocortex and hippocampus (21,23). The four separate genes (HCN1-HCN4) encoding h-channels in rodents have been cloned and h-channels encoded by each of the isoforms have different biophysical characteristics and are differentially distributed in the brain (32). HCN1 and HCN2 are the main brain isoforms, with HCN1 predominantly localized in the neocortex and hippocampus (particularly in CA1) and HCN2 predominant in the thalamus (24). HCN3 is only rarely expressed in the brain and HCN4 is mainly found in the thalamus together with HCN2 (24). Furthermore, HCN1 has faster kinetics but less intracellular cyclic adenosine monophosphate (cAMP)-dependent modulation compared with HCN2 and HCN4 (19,21). The different biophysical characteristics and distribution regarding h-channels in the brain lead them to play a different function in the flow of excitation in the region where they exist.

Since the h-channels are found in the critical regions of the brain, thinking on the link between dysfunction of the h-channels and epilepsy is reasonable and one might view them as an attractive target for antiepileptic drug (AED) action. In this context, experimental studies reported recently focused mainly on dysfunction of the h-channels as a cause of some forms of epilepsy and have shown evidence linking the h-channels and epilepsy (7,20,34). Now, there is molecular evidence supporting the idea of persistent changes in h-channel expressions following hyperthermia-induced febrile seizures (10, 11), kainic-acid and pilocarpine-induced status epilepticus (5,20), and lesion in the entorhinal cortex-induced epilepsy (5) and development epilepsy models in rodents (17,19). However, these studies have demonstrated various expression patterns, up- and/or down-regulation with respect to the same h-channel in the same hippocampal region and neocortex, suggesting that the h-channels might give a different response regarding the expression pattern in case of different seizure induction. Although there have been an increasing number of experimental studies, there is little human data linking the h-channels and epilepsy and no human epilepsy has been associated with HCN mutations so far. There has been only one human study which showed lower HCN mRNA signals throughout the hippocampus but HCN1 mRNA was up-regulated in dentate gyrus cells in the hippocampus with the most severe cell loss (4). Nevertheless, studies published so far have supported the notion that there might be an association between h-channelopathy and epilepsy. As far as we are concerned, there is no laboratory study demonstrating HCN gene expression during the prenatal period during which dysfunction in neuronal network function in the hippocampus might contribute to postnatal epilepsy. Therefore, we conducted an experimental study in which the

prenatal epileptogenesis was induced by giving intrauterine radiation and expressions of HCN1-HCN2 genes were studied in hippocampal CA1 through CA4 regions. In the in utero radiation model of rats, hippocampal dysgenesis has been clearly shown (16,18) which was the main reason that why we used this model in our study.

MATERIALS and METHODS

All the steps in this experimental study were approved by the Istanbul University Animal Care Committee according to the National Institutes of Health Guidelines.

Animals and epileptogenicity

Ten time-pregnant Wistar albino rats and their litters were included in this study. The day in which insemination was detected was designated as day 0 (E0). The radiation was administered on day E17 in which the hippocampal migration is thought to be occur. All animals were maintained on a 12-h light/dark cycle with food and water available ad libitum. Ten pregnant rats were divided into experimental (n = 8) and control (n = 2) groups. The radiation procedure was done according to the protocol described previously. In brief, following the anaesthesia induction (0.2 ml ketamin IM injection to the right posterior leg), the pregnant rats (experimental group) were placed on an appropriate place in supine position. By using Cobalt irradiator, the rats were exposed to a dose of 225 cGy. A total of 4 pregnant rats then had to be excluded from the study due to abortus (n = 3) and one was found not to be pregnant during the study period. Twelve pups from the experimental group survived for 2 months after which they were used to study gene expression. The 2 other pregnant rats (control group) received the same dose of anaesthesia but were not exposed to irradiation, and their 5 pups were used as age-matched controls. Thus 12 and 5 pups served as subjects for this study from the experimental and control groups, respectively. At the age of 2-months, rats were killed by cervical dislocation and the whole brains were rapidly dissected. From each hemisphere, coronal slices were taken under the microscope by one of the author (G.E.) of this paper from the Department of Histology. The hippocampus in the coronal slices was divided according to the regions (CA1 through CA4) and was placed into different tubes which were then placed immediately into the liquid nitrogen tank. In the experimental group, one right (in one pup) and one left (in another pup) hippocampi were damaged unintentionally during the dissection and were excluded from the study. Thus, a total of 32 hippocampi (128 tissues regarding CA1-CA4) from the total of 17 pups [experimental (12 pups, 22 hippocampi and 88 tissues regarding CA1-CA4) and control (5 pups, 10 hippocampi and 40 tissues regarding CA1-CA4)] were evaluated. All tissues were stored in liquid nitrogen (-80 °C) until they were assayed.

Histopathological examination

During assaying, the brains were serially sectioned in the coronal plane by using a cryostat at 50 Ìm and then thaw-mounted onto silane-coated slides. Hematoxylin and eosin (H & E) staining was performed for histological examinations. Stained sections from all animals were analyzed qualitatively according to the following criteria: (a) the presence of cortical neuronal clustering, (b) persistence of neurons in the molecular layer, (c) the lack of columnar and layering organization, and/or (d) the presence of neuronal dispersion in any of the hippocampal subfields. Cortical thickness was determined in two different cuts of the frontal lobe, ~ 1 mm anterior to the bregma, 3 mm lateral from the intrahemispheric fissure, and perpendicular to the surface of each animal (16, 18).

RNA extraction and cDNA synthesis

First, the tissues were lyzed by Magna Lyzer (Roche) for 50 seconds at frequency of 6500, after placing the tissues in "Green Bead Roche©" tubes. RNA extraction was performed by using total RNA isolation kit from Favorprep© RNA Extraction Kit (catalogue number FATRK 001). cDNAs were synthesized by Improm RT II© Promega™ kit (catalogue number A3500), from 50 ng total RNA, by using Oligo(dT)15 primer and manufacturer's instructions.

Reverse transcriptase mediated polymerase chain reaction (RT-PCR)

PCR analysis was performed by using Fermentas Taq Polymerase (catalogue number #EP0281) and specific oligonucleotide primer HCN1-rat-3F CAA-GAGAAGTACAAGCAAGTAGAG and HCN1-rat-4R TGGAACACCTCAAATCTCAG for HCN1, HCN2-rat-right GTTGAAGTTGTCCACGCTCA, HCN2-rat-left CTGCGTGAGGAGATTGTGAA for HCN2. b-actin-left CCTAGAAGCATTTGCGGTG-

CACGATG, b-actin-right TCATGAAGTGT-GACGTTGACATCCGT were used for amplifying beta-actin gene's mRNA. The conditions were; 95°C for 2 minutes followed by 37 cycles of 95°C for 30 seconds, 55°C for 1 minute, 70°C for 1 minute and a final cycle at 70°C for 5 min. Due to high sample size, we normalized the band intensities for each gene by using the median value of a triplicate loaded (in each gel) abundant PCR product. In addition, a second sample-wise normalization was done for HCN1 and HCN2 gene products by using actin mRNA levels. All of the PCRs were performed in triplicate. The band intensities were digitalized by using image J software version 1.33 of NCBI (http://rsb.info.nih.gov/ij/).

Statistical Analysis

Statistical analyses were performed by using the SPSS software version 12.0. Data are reported as the mean ± standard error of the mean. The non-parametric "Mann-Whitney U test" for non-parametric and "Student's t test" for parametric variables were used and if "p" was found to be <0.05, it was accepted as significant.

RESULTS

Neither behavioral nor motor seizures were recorded in any rats exposed to intrauterine irradiation during the study period. During the brain dissection, each rat's brain was noted to be smaller than those of each rat's brain in the control group (Table I). Furthermore, with this experimental model, histopathological examination showed neuronal dispersion in the hippocampus of the rats exposed to intrauterine irradiation (Figure 1A and B).

HCN Gene Expressions

Table II demonstrates mRNA expression levels of HNC1 and HCN2 genes in each subfield in the hippocampus of the rats in the experimental and control groups. The results were normalized by using mRNA level of beta actin and quantified using densitometry analysis by image-j program, as detailed in methods section.

Table I. Weights[a] related to each hippocampal subregion in the experimental and control rats.

	CA1	CA2	CA3	CA4
Experimental	6.16	6.16	6.03	6.1
Control	15.23	13.93	14.23	14.26
"P"	>0.05	>0.05	>0.05	>0.05

[a]Weights are expressed as "mg."; CA: Cornu ammonis.

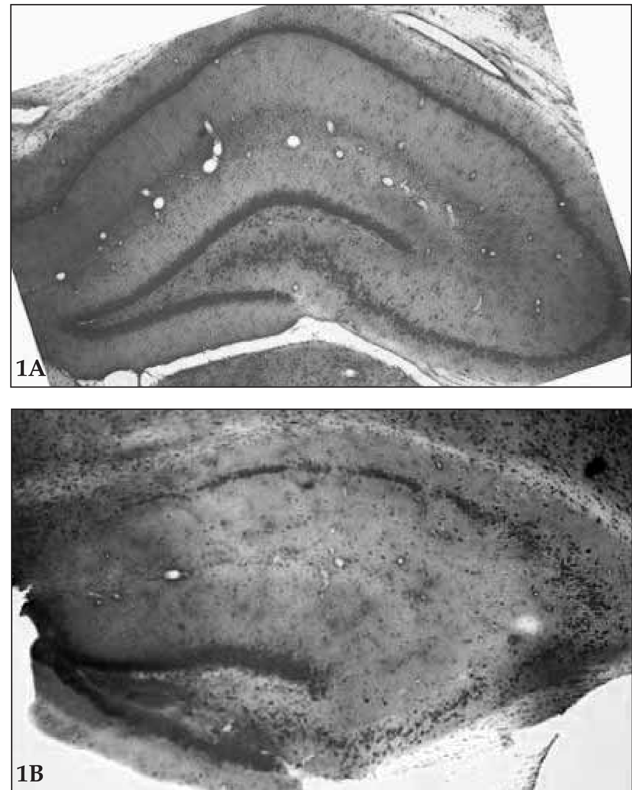

Figure 1: *Histological findings (H&E staining) in the hippocampi in both normal (**A**) and experimental groups (**B**). Presence of neuronal dispersion in pyramidal neurons (CA1 through CA4) is marked compared with controls.*

Cornu ammonis 1 (CA1)

In five tissues, neither HCN1 nor HCN2 gene expressions were detected in experimental group. Four tissues expressing actin did not show HCN1 gene expression. In remaining 13 tissues, the mean HCN1 gene expression was found to be 0.30 ± 0.07 in experimental group whilst it was 0.43 ± 0.11 in controls. However; the difference was not statistically significant between the two groups (p<0.05). When considering HCN2 gene expression, eight tissues normalized by actin did not show HCN2 expression and the mean value in the remaining 14 tissues was 0.52 ± 0.14. Comparison with the controls did not show significant difference with respect to HCN2 (p<0.05). In general, reduction in both HCN1 and HCN2 gene expressions were noted in CA1 subfield of the hippocampus compared with the controls (Figure 2).

Table II. Expression of hyperpolarization-activated cyclic nucleotide-gated channel genes in dysplastic and normal pyramidal neurons.

	CA1		CA2		CA3		CA4	
	HCN1	**HCN2**	**HCN1**	**HCN2**	**HCN1**	**HCN2**	**HCN1**	**HCN2**
Exp (23)	0.30±0.07	0.52±0.14						
Ctr (6)	0.43±0.11	0.65±0.14						
Exp (12)			0.87±0.56	0.21±0.0				
Ctr (2)			0.38±0.17	0.17±0.07				
Exp(25)					0.29±0.05	1.04±0.34		
Ctr (17)					0.39±0.09	2.08±0.68		
Exp(21)							0.31±0.06	0.39±0.09
Ctr (5)							0.28±0.08	0.65±0.24

CA: Cornu ammonis; Exp: Experimental group; Ctr: Control group; HCN: Hyperpolarization-activated cyclic nucleotide-gated channel. Values are presented mean ± standard error of mean.

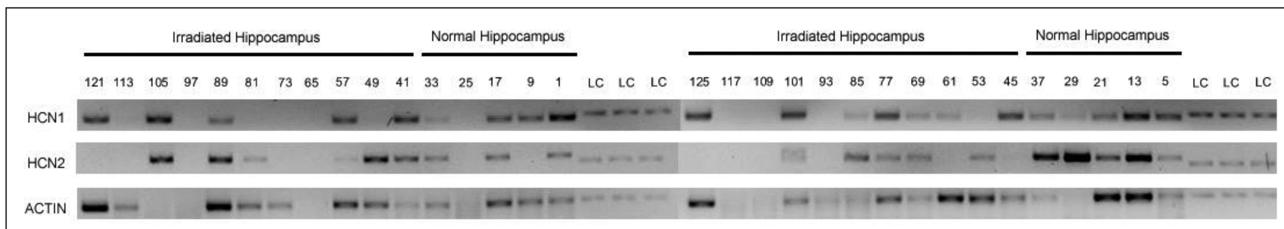

Figure 2: Gel photographs of expression of HCN 1, HCN 2 and B-Actin genes in CA 1 region. The numbers designate the tissue code. (LC: loaded PCR product for normalization).

Cornu ammonis 2 (CA2)

Neither HCN1 nor HCN2 gene expressions were noted in 12 tissues in the experimental group. Eight tissues expressing actin did not show any HCN1 expression. The mean values in remaining 10 tissues that showed HCN1 gene expressions in the experimental group were 0.87 ± 0.56 and 0.38 ± 0.17 in the controls. The difference was not statistically significant ($p<0.05$). In 15 tissues that expressed actin, no HCN2 gene expressions were noted. The remaining 7 tissues in the experimental group showed 0.21 ± 0.0 as mean value. Comparison with the control group did not show statistically significant difference regarding HCN2 ($p <0.05$). In CA2 subfield, both HCN1 and HCN2 gene expressions showed a tendency to increase, being more in HCN1 gene expressions.

Cornu ammonis 3 (CA3)

Six tissues expressing actin did not show any HCN1 gene expression. HCN1 gene expressions normalized by actin in the experiment and control groups were 0.29 ± 0.05 and 0.39 ± 0.09, respectively but the difference did not reach significance ($p < 0.05$). Seven tissues expressing actin did not show any HCN2 expression in the experimental group. HCN2 gene expressions in the remaining 15 tissues in the experimental group (1.04 ± 0.34) decreased compared with the controls (2.08 ± 0.68) but the difference was not statistically significant ($p<0.05$). In CA3, we found that gene expressions decreased compared to controls, being more in HCN2 gene expressions.

Cornu ammonis 4 (CA4)

In 3 tissues, neither HCN1 nor HCN2 gene expressions were noted at all. Eight tissues expressing actin did not show HCN1 gene expression. The mean value in the remaining 11 tissues in the experimental group was 0.31 ± 0.06 and 0.28 ± 0.08 in the controls. The difference was not statistically significant ($p<0.05$). Considering HCN2

expression in CA4, 8 tissues expressing actin had no HCN2 gene expressions. Comparison the two groups with respect to HCN2 gene expressions normalized by actin did not reach significance ($p<0.05$). In CA4 subregion, HCN1 showed a trend to increase while HCN2 gene expressions showed opposite pattern of expression compared with the controls.

DISCUSSION

This study is the first to show HCN1 and HCN2 gene expressions in the dysplastic hippocampus exposed to irradiation in prenatal period. The important finding of the present study is that both HCN1 and HCN2 gene expressions have decreased in CA1 and CA3 sub-regions in the hippocampus, both of which have been shown to play an important role in MTLE with HS. On the other hand, both genes' expressions were increased in CA2 but in CA4, they showed different patterns of expression: HCN1 was increased while HCN2 decreased.

Accumulating evidence during recent years suggests that there might be an association between h-channels and epilepsy. The linking between h-channelopathy and epilepsy was first established by Chen and his colleagues (10, 11). By using novel rat model of complex partial seizure induced by hyperthermia in immature rodent pups, they showed that the prolonged hyperthermia-induced seizure caused persistent increased inhibition of CA1 in the pyramidal neurons and this inhibition was associated with decrease of seizure threshold. In this study, both Ih and GABA-mediated inhibitions were enhanced at the soma of hippocampal pyramidal neurons which led to increased action potential firing after an inhibitory postsynaptic potential, thus producing a net pro-excitatory effect. Although the animals did not have subsequent seizure in this model, their findings suggest that h-channels in the hippocampal pyramidal neurons may have a role in lowering the seizure threshold. The molecular evidence supporting the idea that alterations in h-channels after prolonged febrile seizure came from Brewster, et al. (6) who examined HCN mRNA levels at varying time points after febrile seizure in rats. Levels of HCN1 mRNA which encodes fast activating, slow conductance channels, was significantly reduced 1 week after seizure induction in CA1 and this alteration was found to be site specific. In contrast to HCN1 mRNA, the levels of HCN2 mRNA, encoding slower activating-deactivating channels, were increased in CA1 and CA3 regions in the hippocampus. Thus, increased Ih measured at 1 week after seizure induction actually reflects increased transcription of the HCN2 gene. Furthermore, they demonstrated that seizures rather than hyperthermia per se alter HCN expression in a duration-dependent manner. However; in contrast to above study (6), reduction in HCN1 protein rather than mRNA expression was demonstrated in neocortical and hippocampal neurons of epileptogenic tissue in an animal (WAG/Rij rats) model of absence seizures (17, 36). They suggest that either a post-transcriptional or post-translational deficit may influence the amount of HCN1 protein or Ih reduction was found to be associated with induction of the burst discharges. The switching from fast to slow kinetics (from HCN1 to HCN2) has also been demonstrated in an animal model in which the entorhinal cortex was injured (5). The HCN1 gene was down-regulated throughout the hippocampus immediately after the entorhinal cortex was lesioned, which caused decrease in detectable HCN1 protein and Ih in Mossy fibers and interneurons of the hilar region. Decrease in Ih can cause a decrease in activation of inhibitory neurons, which in turn, reduces inhibition of granule cells, leading to a hyperexcitability of the dentate gyrus, which contributes to acute seizures. The concept that seizure-induced down-regulation of h-channels in the hippocampus was further supported by a kainite-induced status epilepticus model in rats (34). In this model, reduction in both HCN1 and HCN2 protein levels together with acute decrease (24-hour post status) in Ih, was shown in the entorhinal cortex layer III pyramidal neurons. This decrease was associated with EEG abnormalities over the entorhinal cortex. They suggest that the reduction in Ih was associated with the reduction in h-channels which caused dramatic neuronal hyperexcitability. The persistent reduction in Ih one week after status explains that posttranslational change may have also been involved in the loss of channel function. The results of this study seem different than those of Chen, et al's group (10, 11) and these discrepancies may be explained by the methodological differences and merit further studies. Contribution of h-channel derangements per se to epilepsy has been demonstrated well in global knockout of the HCN2 gene in mouse models (12, 19). In this model, HCN2 deficient mice displayed frequent bilaterally

synchronous spike-wave discharges, the hallmark of absence epilepsy. The resting membrane potential of thalamo-cortical neurons in HCN2 deficient mice were more hyperpolarized than those of the wild type cells. Furthermore thalamo-cortical neurons in which HCN2 predominate showed dramatic reduction of Ih and heightened burst firing compared to wild type mice. The results could be interpreted as the lack of HCN2 decreases seizure threshold in the thalamo-cortical neurons which, in turn, may lead to the predisposition to absence epilepsy. Interestingly, in this study no significant differences were found in the distribution pattern of HCN1, HCN3, and HCN4 between wild type and HCN2 deficient mice. These results suggest that seizure by itself does not modulate the expression of other HCN channel genes. Another rodent model of absence epilepsy induced in HCN1 deficient (8, 17) mice showed similar results, suggesting absence epilepsy could be associated with dysfunction of HCN1 and HCN2 subunits. Important evidence showing the correlation between h-channelopathy and onset of spontaneous seizure came from the rat pilocarpine model of epilepsy (20). During the acute period, 1 week after pilocarpine induction, HCN1 and HCN2 channel function decreased in CA1 pyramidal neurons with a hyperpolarized shift in activation of Ih. Furthermore, Ih decreased further in pyramidal neuron dendrites in the chronic period (3-5 weeks), suggesting that h-channelopathy occurs in the acute phase and persists to the chronic period as the animals experience recurrent seizure. In the chronic phase, only HCN1 channels were found to be decreased, suggesting that HCN2 expression reduced in acute phase had returned to normal. Suppression of seizures with phenobarbital showed that changes in Ih during acute phase were dependent on spontaneous seizure activity but loss of Ih and expression of h-channels were independent of spontaneous seizures. Thus it seems that hyperexcitability mediated by h-channels contributes to the spontaneous seizures.

Taken together, results clearly suggest that epilepsy might be associated with loss or down-regulation of Ih, which exerts anti-epileptic function. Linking between h-channelopathy and epilepsy has also been shown in a human study (4). In this study HCN1 expression pattern, at both mRNA and protein levels were shown to be significantly decreased in surgical specimens with severe sclerosis compared with autopsy and non-hippocampal sclerosis specimens. More importantly, some specimens with HS showed robust mRNA expression of HCN1 in dentate gyrus granule cells and this up-regulation was considered compensatory response by unaffected neurons due to ongoing seizure in an attempt to reduce excitation. Although controversial, some studies demonstrating up-regulation of h-channels by some AEDs showed promising results (27). In these studies it has been demonstrated that pharmacological increase in Ih depolarizes resting potential, moving it towards threshold for action potential firing, thus increase inhibition on excitatory inputs.

Our results confirmed previous studies that epileptogenic tissues, the hippocampus in our study, showed alterations in HCN1 and HCN2 genes that were found to be decreased in the CA1 and CA3 sub-regions in the hippocampus. However; they increased in CA2 and CA4. Since we did not find significant differences regarding HCN1 and HCN2 gene expressions between the animals exposed to high dose irradiation in utero and the controls, one may speculate that the dose that we gave was not enough to make dysplastic changes and to alter the gene expressions. However; our model caused severe dyplasia in the brain including the hippocampus and it was confirmed by histopathological examination, which is in agreement with the study by Kellinghaus et al (16). Thus, we can speculate that insignificant differences between the two groups might be due to compensatory increase by unaffected pyramidal neuronal cells. Furthermore, depending on our results, it is possible that marked alteration in HCN1 and HCN2 channel gene expressions may require seizure activity as shown in some previous studies (7,10,11,20,34) though we did not observe any seizure activity during the study period. With the same context, the underlying mechanism that why the animals did not show seizure activity can partly be explained due to the fact that Ih did not show alteration in biophysical features which keep the membrane potential stable, thus inhibiting the hippocampal neuron progression to hyperexcitable state. When we consider that dysplastic tissue is a well-proven pro-epileptogenic, trend to decrease in h-channels in CA1 and CA3 pyramidal neurons in our study led us to think that there might be a negative correlation between h-channels and epilepsy; lower the Ih, higher the possibility to have

seizure. The findings from outbursting studies including ours clearly showed that there are alterations in h-channel expressions in some forms of epilepsy shown in animal models but could not show that an observed changes represent causation or compensation regarding epileptogenesis.

CONCLUSION

Our results showed that dysplastic changes induced by in utero irradiation at a dose of 225 cGy may not be enough to produce marked alterations in h-channels per se. However; it is clear that HCN1 and HCN2 gene expressions decreased in CA1 and CA3 pyramidal neurons which play an important role in the contribution to mesial temporal lobe epilepsy with hippocampal sclerosis. The rationale for how these changes contribute to epileptogenesis in dysplastic tissues still requires further studies.

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