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Functional rundown of GABA_A receptors in human hypothalamic hamartomas

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Abstract

Objective—Human hypothalamic hamartomas (HH) are highly associated with treatment-resistant gelastic seizures. HH are intrinsically epileptogenic, although the basic cellular mechanisms responsible for seizure activity are unknown. Altered gamma-aminobutyric acid (GABA) function can contribute to epileptogenesis in humans and animal models. Recently, functional GABA_A receptor (GABA_AR) rundown has been described in surgically-resected human temporal lobe epilepsy tissue. We asked whether functional GABA_AR rundown also occurs in human HH neurons.

Methods—GABA_AR-mediated currents were measured using perforated patch-clamp recordings in single neurons acutely dissociated from surgically-resected HH tissue. In addition, functional GABA_AR were expressed in *Xenopus* oocytes after microinjection with membrane fractions from either HH or control hypothalamus, and were studied with two-electrode voltage-clamp recordings.

Results—Perforated patch-clamp recordings in dissociated HH neurons showed that repetitive exposure to GABA (5 consecutive exposures to 0.1 mM GABA with 1 sec duration and at 20 sec intervals) induced a time-dependent rundown of whole-cell currents in small HH neurons, while large HH neurons showed much less rundown using the same protocol. Functional rundown was not observed in HH neurons with repetitive exposure to glycine or glutamate. Two-electrode voltage-clamp recordings (6 consecutive exposures to 1 mM GABA with 10 sec duration and at 40 sec intervals) induced GABA-current rundown in *Xenopus* oocytes microinjected with HH membrane proteins, but not in the oocytes expressing hypothalamic membrane proteins derived from human autopsy controls. Functional rundown of GABA-currents was significantly attenuated by intracellular application of adenosine triphosphate (ATP) or the non-specific phosphatase inhibitor, okadaic acid.

Interpretation—Neurons from surgically-resected human HH demonstrate functional rundown of GABA_AR-mediated transmembrane currents in response to GABA agonist exposure. Rundown may be a marker for impaired GABAergic function and a contributing mechanism for seizure genesis within HH tissue.

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Keywords

γ -Aminobutyric acid (GABA); GABA_A receptor; rundown; hypothalamic hamartoma; *Xenopus* oocytes; patch-clamp; epilepsy; gelastic seizures

Introduction

Hypothalamic hamartomas (HH) are developmental malformations of the ventral hypothalamus, associated with diverse neurological and endocrinological symptoms, including epilepsy, cognitive impairment, behavioral disturbances and central precocious puberty.^{1–6} HH is associated with gelastic (laughing) seizures, often beginning in early infancy, that are refractory to standard anti-epilepsy drugs (AEDs).^{2,7–9} Gelastic seizures arising from HH are an important human model of sub-cortical epilepsy.^{10,11} Seizure recordings from electrodes placed directly into the HH have demonstrated that HH tissue is intrinsically epileptogenic for gelastic seizures.^{12–15} However, the basic cellular and molecular mechanisms responsible for epileptogenesis within HH are unknown. Detailed understanding of these mechanisms may lead to novel treatment strategies, or insights into the pathogenesis of other forms of epilepsy.

Recent evidence suggests that functional rundown of the GABA_AR may be a mechanistic component of human temporal lobe epilepsy (TLE)^{16–17} and epilepsy associated with human cortical dysplasia¹⁸. *Xenopus* oocytes micro-injected with membrane proteins from surgically-resected TLE tissue exhibit functional rundown during repetitive exposure to GABA.^{16,17,19} GABA_AR functional rundown may be an important mechanism in disrupting inhibitory function, contributing to excitation-inhibition imbalance and resulting in epileptic seizures. Functional rundown appears to be mediated by GABA_AR desensitization (or internalization) resulting from dephosphorylation of receptor subunits.^{20,21} Consistent with this observation, phosphatase inhibitors significantly reduce GABA_AR rundown in human TLE tissue, potentially representing a novel therapeutic approach to preventing seizures.^{22,23}

HH most likely originate as a result of aberrant development within the ventral hypothalamus, although the specific cellular lineage of these lesions is unknown. HH lesions associated with central precocious puberty (CPP) are attached to the tuber cinereum,⁶ while those associated with epilepsy are attached more posteriorly near the mammillary bodies.^{24,25} Microscopically, HH tissue consists of normal-appearing glia and supporting cells, with clusters of small but mature neurons.^{26,27} Smaller numbers of large neurons are also present, resembling the principal neurons within hypothalamic nuclei, and demonstrate positive immunoreactivity to gonadotropin-releasing hormone (GnRH) and other hypothalamic markers.^{28–31} The hypothalamus is the ideal control tissue for experimental study of HH. However, while treatment may include resection of the HH, there is a high premium placed upon avoiding injury to the adjacent hypothalamus, and even partial resection of normal adjacent tissue is not acceptable surgical practice. An animal model for HH has not been developed. Accordingly, there is a need to utilize innovative techniques to enable controlled experimentation with HH tissue, particularly for electrophysiological methods that use freshly-resected tissue.

Previous work has demonstrated that (1) there are at least two neuron phenotypes in HH tissue, the *small* and the *large* HH neurons,^{32,33} (2) small HH neurons are GABAergic and exhibit intrinsic pacemaker-like spontaneous firing,³² (3) small HH neurons project synaptic connections to both large and other small HH neurons,³⁴ and (4) large HH neurons are functionally immature, with depolarization and increased firing in response to GABA

agonist administration.^{35,36} This line of evidence suggests that an abnormality related to GABAergic transmission could play a role in HH epileptogenesis. In this report, we examined whether functional GABA_AR rundown occurs in HH tissue.

Materials and Methods

Clinical profile of study subjects

Informed consent for the use of resected tissue for research purposes was obtained according to protocols approved by the Institutional Research Board (IRB) of the Barrow Neurological Institute, St. Joseph Hospital and Medical Center, Phoenix, AZ.

Tissue samples were obtained from 23 patients treated at our institution between June 2003 and September 2008. There were 13 females (57%). The mean age at the time of surgery was 10.3 years (median age 6.0 years; range 0.8 – 36.8 years). All patients had treatment-resistant epilepsy, with onset of gelastic seizures at 12 months of age or less in 21 (91%). At the time of surgery, 21 (91%) had multiple daily seizures.

Co-morbidity included developmental delay (full-scale intelligence quotient or estimated developmental quotient <70) in 6 (26%), a history of CPP in 9 (39%), and Pallister-Hall syndrome (OMIM 146510) in one (4%). The mean HH tissue volume was 4.28 cm³ (median 2.62 cm³; range 0.22 – 38.25 cm³) according to the previously published method.²³

Human control tissue

For the oocyte microinjection controls, post-mortem hypothalamic tissue derived from normal human donors was obtained from the Harvard Brain Bank (n = 2; 1 male) and the Human Brain and Spinal Fluid Resource Center at the West Los Angeles Healthcare Center (n = 2; both males). The mean age was 59.3±7.2 years (range 47.0 to 78.0 years). Brain tissue was collected (frozen) within 4 hours after death.

Freshly dissociated neurons from HH tissue

Preparation of acutely dissociated single HH neurons was performed according to previously published methods.^{32,35,37,38} Briefly, HH tissue obtained at the time of surgical resection was placed immediately into ice-cold dissection solution (2–4°C) that contained (in mM): 136.7 NaCl, 5 KCl, 0.1 Na₂HPO₄, 0.2 KH₂PO₄, 9.84 HEPES, 16.6 D-glucose, and 21.9 sucrose; pH 7.3; 330 mOsm; oxygenated with 100% O₂. Tissue sections of 350–400 μm were cut using a VF-100 sliding microtome (Precisionary Instruments Inc., Greenville, NC), and were transferred immediately to a recovering bath containing (in mM): 125 NaCl, 2.5 KCl, 1.2 NaH₂PO₄, 1.2 MgCl₂, 2.4 CaCl₂, 26 NaHCO₃ and 11 glucose for at least 1 hour before dissociation (at 21–22°C). Tissue sections were then treated with 4–6 mg/ml papain (Sigma Chemical Co., St. Louis, MO) for 50–60 minutes (at 31°C). Each tissue section was then mechanically dissociated using fire-polished micro-Pasteur pipettes with oxygenated standard extracellular solution containing (in mM): 150 NaCl, 5 KCl, 1 MgCl₂, 2 CaCl₂, 10 glucose, and 10 HEPES; pH 7.4 (with Tris-base). Isolated single neurons usually adhered to the bottom of the dish within 30 minutes and were viable for 2–6 hours.

Patch-clamp recordings in dissociated HH neurons

Perforated-patch whole-cell recordings, combined with a U-tube fast application system, allowing for both quick application and removal of drug, were employed in dissociated HH neurons using previously described techniques.^{32,35,38} The perforated-patch pipette solution contained (in mM): 150 CsCl, 4 MgCl₂ and 10 HEPES, adjusted to a pH of 7.2 using Tris-OH (CsCl electrode). Amphotericin B or gramicidin was dissolved in DMSO (40 mg/ml for amphotericin B and 10 mg/ml for gramicidin as a stock) and diluted with internal

(patch-pipette) solution to a final concentration of 200–250 µg/ml (amphotericin B) or 70–100 µg/ml (gramicidin) immediately before use. GABA-induced currents were recorded using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Whole-cell access resistance less than 60 MΩ was accepted for experiments. In 25 HH neurons that exhibited GABA current rundown, the average whole-cell access resistance was 30.3 ± 2.4 MΩ. The series resistance was not compensated in this study. Typically, data were acquired at 10 kHz, filtered at 2 kHz, displayed and digitized on-line (Digidata 1322 series A/D board, Axon Instruments). All experiments were performed at room temperature. All drugs used in this study were purchased from Sigma-Aldrich (St. Louis, MO) and Tocris Cookson, Inc (Ellisville, MO).

Human tissue membrane preparation, injection procedures, and two-electrode voltage-clamp recording from oocytes

The protocol for the preparation of *Xenopus* oocytes was approved by the Institutional Animal Care and Use Committee of the Barrow Neurological Institute, St. Joseph's hospital and Medical Center, Phoenix, AZ.

Membrane proteins from HH tissue and normal human hypothalamus were prepared and injected into oocytes according to published protocols.¹⁹ In brief, tissue frozen in liquid nitrogen was homogenized in glycine buffer by using a glass homogenizer. The filtrate was centrifuged for 15 minutes at $9,600 \times g$ in an Eppendorf Centrifuge 5417R–F45-30-11 rotor. The supernatant was then centrifuged for 2 hours at $100,000 \times g$ in a Beckman L-7 Ultracentrifuge SW61 Ti rotor at 4°C. The pellet was washed, re-suspended in 5 mM glycine, and utilized immediately, or frozen at -80°C for later use. The incubation solution for *Xenopus* oocytes contains (in mM): 82.5 NaCl, 2.5 KCl, 1 MgCl₂, 1 CaCl₂, 1 Na₂HPO₄, 2.5 sodium pyruvate, 0.6 theophylline and 5 HEPES, adjusted to pH 7.5 with 2M NaOH at 24–25°C, sterile filtered with a NALGENE filter (Nalge Nunc International Corporation) with added antibiotics (penicillin – streptomycin – neomycin solution, P4083).

GABA_A-currents were elicited by 1 mM GABA. The GABA-current rundown was defined and recorded as previously described.¹⁸ The oocyte Ringer solution (OR2) has the following composition (in mM): 92.5 NaCl, 2.5 KCl, 1 MgCl₂, 1 CaCl₂ and 5 HEPES, adjusted to pH 7.5 with 2M NaOH at 24–25°C.

Statistics

The results were expressed as mean $\pm$ SEM. Two-tailed Student's t-test was used to determine statistical differences (significance $p < 0.05$). Significant p values for each experiment are provided unless $p < 0.001$.

Results

Functional rundown of GABA_A-receptors in dissociated single HH neurons

Our initial experiments were designed to determine if GABA_AR-mediated current (GABA-current) rundown occurs in dissociated small and large HH neurons. In 21 small HH neurons dissociated from 11 patients (age = 7.8 ± 2.0 years), repetitive exposure with 100 µM GABA (5 exposures of 1 sec duration at 20 sec intervals) induced a progressive decline in the peak amplitude of the GABA-current. The normalized ratio of 5th/1st (1st as 100%) of peak amplitude of the GABA-current was $60.6 \pm 3.8\%$ ($n=21$, $p < 0.001$). In 7 large HH neurons dissociated from 7 HH patients (age = 7.5 ± 2.6 years), the same experimental protocol induced much less decline of GABA-current (Fig. 1B). The normalized ratio (5th/1st) of peak amplitude for GABA-current was $81.8 \pm 2.9\%$ ($n=7$, $p=0.293$). The difference of the ratio (5th/1st) of GABA-current between small and large HH neurons was significant

($p=0.012$). Figure 1C summarizes our finding on functional rundown of GABA-currents in small and large HH neurons.

Functional rundown in HH neurons is specific to GABA_A-receptors

We asked whether functional rundown of ligand-gated transmembrane currents is specific to GABA by examining the effect of repetitive exposure to other receptor agonists (glycine and glutamate) in small HH neurons. Figure 2A shows GABA-current rundown in 7 small HH neurons tested (dissociated from 3 HH patients). In these same HH neurons, repetitive exposure to 100 μ M glycine (Fig. 2B, $n=7$) and 1 mM glutamate (Fig. 2C, $n=3$) did not result in functional rundown of GABA currents. The normalized ratio (5th/1st) of peak current amplitude was $63.1 \pm 6.1\%$ for GABA-current ($n=7$, $p<0.001$), $99.2 \pm 5.0\%$ for glycine-current ($n=7$, $p=0.943$) and $107.1 \pm 5.2\%$ for glutamate current ($n=3$, $p=0.281$), respectively. These results suggest that functional rundown of GABA_AR mediated current is a relatively specific event in small HH neurons.

Comparison of functional rundown of GABA_A-receptors between HH and normal human hypothalamus

To determine if functional GABA_AR rundown is also present in normal human hypothalamus, we employed a *Xenopus* oocyte microinjection approach reported by Miledi and colleagues.¹⁹ In these experiments, the oocytes injected with either HH or normal human hypothalamic membrane fractions were maintained in incubation solution on a shaker at 18° C. As early as 12 hours after membrane injection, GABA_AR-mediated currents can be elicited by 1 mM GABA, suggesting that functional GABA_ARs have been expressed on the surface of the oocytes, consistent with previous reports.^{16,23}

In 160 oocytes (from 25 frogs) injected from 8 HH patients, the mean peak amplitude of GABA_AR-mediated current was 100 ± 65 nA at a holding potential of -60 mV. With the rundown protocol utilized for oocytes (6 consecutive exposures to 1 mM GABA for 10 sec duration at 40 sec intervals),¹⁷ the peak amplitude of the GABA-currents in the oocytes injected with HH membrane fractions declined in a time-dependent manner (Fig. 3A). After a 2 min washout, the reduced peak currents recovered to pre-exposure baseline values (Fig. 3A). The normalized ratio (6th/1st) of GABA-current was $61.5 \pm 0.8\%$ ($n=160$, $p<0.001$).

GABA_AR-mediated currents in the oocytes injected with membrane protein fractions derived from normal human autopsy controls ($n=4$ subjects) showed less rundown (Fig. 3B, $n=56$). The normalized ratio (6th/1st) of GABA-current was $76.8 \pm 0.7\%$ ($p=0.046$). The difference in altered ratio (6th/1st peak amplitude) between HH and control groups is statistically significant ($61.5 \pm 0.8\%$ vs. $76.8 \pm 0.7\%$, $p<0.001$). Figure 3C compares GABA-current rundown between HH and control tissues.

Functional rundown of GABA_AR in HH and normal control tissue is not age-dependent

Considering the age difference between the cohort of HH patients included in the oocyte microinjection experiments (mean age 28.0 ± 8.2 years, $n=8$) and controls (mean age 59.3 ± 7.2 years, $n=4$, $p<0.001$), we analyzed the possible influence of age on GABA-mediated functional rundown. There was no significant difference in GABA-current rundown versus age within either the HH or control cohorts (Fig. 4A, B). We also examined functional rundown between the HH and control cohorts on an age-matched basis. In 27 oocytes expressed from 3 HH patients (41.3 ± 8.0 years), GABA induced more functional rundown in comparison to age-matched controls (43 oocytes expressed from 2 controls, 48.0 ± 1.0 years; Fig. 4C). While the difference in age is not significant ($p=0.561$), the difference in the peak amplitude ratios (6th/1st) of GABA-currents is significantly different ($64.6 \pm 1.7\%$ for HH group; $75.3 \pm 1.1\%$ for control group; $p<0.001$). These results suggest that the

difference in functional rundown of GABA-current between the HH and normal hypothalamus control groups is related to pathology rather than age.

Intracellular ATP eliminates GABA_A-receptor rundown in small HH neurons and microinjected oocytes

Functional run-down of GABA_AR-mediated current is related to the intracellular ATP level. 35-36 To test the effects of intracellular ATP on GABA_AR rundown in small HH neurons, we added 5 mM ATP-Mg²⁺ into the recording pipette solution. We initially performed the rundown protocol with acutely-dissociated small HH neurons while utilizing the perforated patch (gramicidin) recording technique, and then converted the perforated patch into conventional whole-cell patch configuration by brief suction (Fig. 5Aa,b). This approach allows ATP-Mg²⁺ and a high concentration of Cl⁻ (using 150 mM CsCl pipette solution) to diffuse into the recorded neuron. After conventional whole-cell recording for 5–10 min, we repeated the rundown protocol, and compared functional rundown before and after ATP infusion. Under perforated patch conditions, repetitive exposure to GABA induced GABA-current rundown (Fig. 5Aa, n=14), while after intracellular infusion of 5 mM ATP-Mg²⁺, functional GABA_AR rundown was significantly reduced (n=4, Fig. 5Ab,c).

Oocytes expressing membrane proteins from HH tissue were injected with 5 mM ATP-Mg²⁺, and GABA_AR-mediated currents were measured after 8–10 minute. Figure 5Ba shows that oocytes with elevated intracellular ATP levels demonstrated significantly less GABA-current rundown (6th/1st ratio 72.8 ± 2.3%; n=13) versus oocytes without injected ATP (6th/1st ratio 58.2 ± 2.2%; n=13; *p*=0.005 between ATP and no ATP groups). These results suggest that intracellular ATP concentrations can mediate GABA-current rundown.

Phosphatase inhibitor okadaic acid (OA) reduces GABA-current rundown in small HH neurons and microinjected oocytes

GABA_AR function, including GABA-induced rundown, is influenced by phosphorylation of the GABA_AR. 39-41 To explore whether phosphorylation affects functional rundown in small HH neurons, the nonspecific phosphatase inhibitor okadaic acid (OA) was applied. In 4 dissociated small HH neurons (from 3 HH patients), bath perfusion of 20 nM OA for 10 min significantly eliminated GABA-current rundown (Fig. 6A). In these same small HH neurons, the normalized ratio (5th/1st) of peak GABA-currents was 39 ± 5% prior to OA perfusion, and 71 ± 9% in the presence of OA (n=4, *p*=0.047).

GABA-mediated rundown was also significantly reduced in oocytes expressing HH membrane proteins, following the intracellular injection of 2 nM OA (Fig. 6B; n=13 oocytes from 4 HH patients). In these same oocytes, the normalized ratio (6th/1st) of GABA-current was 59.2 ± 2.2% prior to OA injection and 68.9 ± 1.6% after 6–8 min of OA exposure (n=13, *p*=0.002). These results suggest that dephosphorylation of GABA_ARs (or closely associated proteins that mediate GABA_AR function) contributes to GABA_AR rundown in HH tissue.

Discussion

Functional GABA_AR rundown in human HH tissue

These findings demonstrate that GABA-mediated functional rundown occurs in surgically-resected epileptogenic HH tissue. In the absence of the availability of surgically-resected normal human hypothalamus, we have utilized *Xenopus* oocytes microinjected with membrane proteins from HH and normal human autopsy controls, and demonstrated that significantly less GABA-current rundown occurred in the control group under our experimental protocol. Small HH neurons (the most abundant neuron in HH tissue³³)

demonstrate a significantly greater degree of functional rundown in comparison to large HH neurons, and this appears to be ligand-specific, as it was observed with GABA administration but not following exposure to either glycine or glutamate. Lastly, GABA-mediated rundown in small HH neurons was attenuated by increased intracellular concentrations of ATP, and was largely reversed by a phosphatase inhibitor (okadaic acid). These findings are consistent with the hypothesis that rundown is mediated by dephosphorylation of the GABA_AR.

The role of GABA_AR rundown in epileptogenesis

Imbalance between cerebral excitation and inhibition caused by functional impairment of GABAergic signaling can result in epileptic seizures.^{39–43} Functional rundown of the GABA_AR is one mechanism by which the inhibitory effects of GABA may be reduced. GABA_AR functional rundown, a phenomenon characterized by exposure-dependent decreases in transmembrane Cl[−] current in response to GABA agonists, has been observed during patch-clamp recordings^{21·40·44·45} under pathological conditions such as ischemia⁴⁶ and epilepsy.^{16·17·19} Recent evidence indicates that functional GABA_AR rundown occurs in human epileptic temporal lobe tissue,^{16·17·19} and human cortical dysplasia,¹⁸ suggesting a possible mechanistic role for GABA_AR rundown in epileptogenesis.¹⁸

Two neuron phenotypes have been identified in surgically-resected HH tissue: the small and the large HH neuron.^{32–35·38·47} Approximately 90% of HH neurons are small neurons, which exhibit immunocytochemical staining consistent with a GABAergic phenotype, and demonstrate intrinsic pacemaker-like firing.³² Conversely, most large HH neurons have a functionally immature phenotype with depolarization and enhanced firing in response to GABA agonist administration.^{35·36·47} Small HH neurons are known to project to large HH neurons³⁴ and may therefore paradoxically excite these neurons, which may mediate the output from HH tissue. Small HH neurons also project to other small HH neurons with inhibitory synapses³⁴ that may act to modulate or perhaps synchronize small HH neuron pacemaker-like firing. This small neuron-large neuron network, driven by GABAergic activity, may be the functional unit for intrinsic seizure genesis within HH.

In this report, we find another electrophysiological difference between small and large HH neurons: small HH neurons exhibit much more GABA_AR rundown than large HH neurons. We hypothesize that the difference of GABA_AR rundown between small and large HH neurons might contribute to epileptogenesis within HH tissue. One possibility is that functional rundown of the GABA-current may alter inhibitory control over small HH neurons, resulting in more net GABA release to large projection neurons (resulting in depolarization and excitation of the large neurons). Alternatively, rundown may enhance synchrony within small neuron clusters. GABA_ARs on large HH neurons exhibit less functional rundown, thereby possibly maintaining a high level of excitatory output from the HH to brain circuitry responsible for expressing gelastic seizures.

GABA_AR rundown as a potential therapeutic target

Although the precise mechanisms are still unclear, the current hypothesis is that functional ligand-gated receptor rundown reflects a change in the tonic activity of an intracellular modulator (or modulators) of receptor-ion channel activity. Intracellular ATP^{21·40·44–46·48} and Ca²⁺⁴⁹ play a role in mediating GABA_AR rundown. Previous studies on wild-type receptors have suggested that an increase (or maintenance) in intracellular ATP levels limits rundown of the GABA_AR by acting as a substrate for protein kinases.^{39·40·47·50} Phosphorylation/dephosphorylation is a common mechanism for regulating receptor-ion channel function.⁵¹

Selective enhancement of inhibitory drive is a potential strategy to modulate cortical hyperexcitability. Many commonly used anti-epilepsy drugs (AEDs) act via the GABA_AR. In this study, we found that an increase of intracellular ATP or pharmacological block of protein phosphatase activity largely reversed GABA_AR rundown in HH neurons, suggesting a novel therapeutic strategy for controlling gelastic seizures arising from HH tissue. It has recently been reported that levetiracetam stabilizes GABA_ARs derived from human epileptic tissue, either by preventing GABA_AR rundown or by accelerating GABA_AR recovery from rundown.^{52–55} The possibility that levetiracetam is a disease-specific therapy for gelastic seizures associated with HH should be addressed with a controlled treatment trial.

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Figure 1.

GABA-current rundown in small and large HH neurons. Under perforated patch recording (amphotericin B, 150 mM CsCl in the pipette solution), GABA was repetitively applied to HH neurons (100 μ M, 1 sec exposure at an interval of 20 sec) as shown in the short horizontal bars above each trace (A and B). C: Comparison of GABA-current rundown between small and large HH neurons. In this and all following figures, the vertical bar represents $\pm$ SEM. Comparison of rundown between 1st trace (as 100%) and subsequent traces: * $p<0.05$, ** $p<0.01$. Comparison of rundown between HH and control: # $p<0.05$, ## $p<0.01$.

Figure 2.

Comparison of functional rundown observed for different ligand-gated channels. Using the same protocol, functional rundown occurred with GABA-induced currents (A) but not with glycine- (B) or glutamate-induced currents (C). D: Comparison of rundown responses for GABA, glycine, and glutamate exposure.

Figure 3.

GABA-current rundown in oocytes injected with HH and control tissue membrane fractions.

A: Typical trace shows an inward current mediated by human HH GABA_AR expressed in oocytes. Under the rundown protocol for oocytes (1 mM GABA, 10 sec exposures at 40 sec intervals),⁵⁵ peak GABA-current declined with repeated exposures. Two minutes after the last GABA exposure, the peak GABA-current response has largely recovered. B. This protocol was repeated with oocytes injected with membrane fractions derived from normal human autopsy controls. C: Cumulative time course of functional rundown in oocytes injected with HH membrane fractions from eight patients (circles; 160 oocytes) and from four normal controls (squares; 56 oocytes). All peak currents were normalized to the first GABA exposure response. Compared to 1st response (trace), subsequent responses (traces 2–6) showed rundown in both HH and control tissues. However, the difference in the peak amplitude rundown response between HH and controls was highly significant ($p < 0.01$ for traces 2–6 indicated by ##).

Figure 4.

GABA-current rundown is not age-dependent. A: In four controls with different ages (range 45.3 to 78 years), there is no significant difference in GABA-current rundown. B: Likewise, there is no significant difference in GABA-mediated rundown in the cohort of HH patients (range 1.1 to 57 years). The number in each open bar in panels A and B indicate the number of oocytes tested for each subject. C: An age-matched comparison between the HH (n=3; mean age 41.7 ± 7.8 years) and control (n=2; mean age 47.2 ± 1.9 years) cohorts. These age-matched results show significantly greater functional rundown in the HH cohort than in control cohort (### $p < 0.01$).

Figure 5.

Increased intracellular ATP prevents GABA-current rundown. A: To compare the effects of intracellular ATP on GABA-current rundown in the same neuron, the rundown protocol was initially performed under perforated patch conditions (gramicidin, 150 mM CsCl and 5 mM ATP-Mg²⁺ in the pipette solution), in which GABA induced an outward current since the gramicidin pore is not permeable to Cl⁻ ion, and thus external Cl⁻ enters the cell during GABA_AR activation. Under this condition, GABA-current rundown occurred (Aa). Brief suction was then applied to convert the recorded neuron to conventional whole-cell mode, and in this way, ATP and Cl⁻ were infused into the cell. After 10 min, the rundown protocol was repeated. Under whole-cell conditions (intracellular 5 mM ATP and 150 mM Cl⁻), Cl⁻ exits the cell in response to GABA, resulting in an inward current, and GABA-currents showed much less rundown (Ab). Ac: Line graph summarizes the data from A and B, and shows that an increase of intracellular ATP-Mg²⁺ significantly prevents GABA-current rundown in dissociated small HH neurons. B: Similar results were also obtained from oocytes injected with HH membrane fractions without (Ba) and with (Bb) injection of 5 mM ATP-Mg²⁺, showing a significant reduction of GABA-current rundown with ATP exposure (Bc).

Figure 6.

Reversal of GABA-current rundown by the non-specific phosphatase inhibitor okadaic acid (OA). In dissociated small HH neurons, GABA-current rundown was reduced after bath application of 20 nM OA for 10 min (panel A; typical tracings are illustrated in the insets; $p=0.047$). This effect was also observed in oocytes microinjected with membrane fractions of HH tissue, and infused with 2 nM OA (panel B; typical tracings are illustrated in the insets; $p=0.002$).