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Pharmacology, Biochemistry and Behavior 82 (2005) 215 – 222
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Male transgenic glycine receptor α_1 (S267Q) mutant mice display a hyperekplexia-like increase in acoustic startle responses

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Received 21 September 2004; received in revised form 13 August 2005; accepted 16 August 2005
Available online 15 September 2005

Abstract

Glycine receptors (GlyR) are ligand-gated ion channels that inhibit neurotransmission in the spinal cord and brainstem, and mutations in GlyR can cause the human disease hyperekplexia, which is characterized by elevated startle responses. Recently, the GlyR α_1S267Q mutation was shown to disrupt normal GlyR function, and knock-in mice harboring this mutation displayed profoundly increased acoustic startle responses and reduced glycine-stimulated the chloride flux [Findlay, G.S., Phelan, R., Roberts, M.T., Homanics, G.E., Bergeson, S.E., Lopreato, G.F., Mihic, S.J., Blednov, Y.A., Harris, R.A. 2003. Glycine receptor knock-in mice and hyperekplexia: comparisons with the null mutant. J Neurosci 23, 8051–8059.]. In this study, a transgenic mouse model expressing this S267Q mutation was evaluated using similar techniques to determine if these mice are similarly affected. Male transgenic mice displayed increased acoustic startle responses. However, decreases in glycine-stimulated strychnine-sensitive radioactive chloride ($^{36}Cl^-$) uptake were not observed in spinal cord and brainstem synaptoneurosomes from transgenic mice. No changes in habituation or prepulse inhibition of startle responses or spontaneous locomotion in response to taurine were observed as a result of presence of the transgene. Consistent with previous studies using immunoblotting and strychnine binding [Findlay, G.S., Wick, M.J., Mascia, M.P., Wallace, D., Miller, G.W., Harris, R.A., Blednov, Y.A. 2002. Transgenic expression of a mutant glycine receptor decreases alcohol sensitivity of mice. J Pharmacol Exp Ther 300, 526–534.], the glycine-stimulated strychnine-sensitive chloride flux of cortical microsacs in transgenic mice confirmed the ectopic expression of transgenic GlyR. These results support both the idea that transgenic expression of the S267Q mutation produces a less dramatic phenotype as compared to the knock-in mouse model as well as the idea that the in vivo acoustic startle test (as compared to the in vitro chloride flux assay) is particularly sensitive to disruptions in GlyR function.

Keywords: GlyR; Mice; Transgenic; Acoustic startle response; Glycine; Taurine; Strychnine-sensitive; Ectopic; Chloride flux

1. Introduction

Glycine receptors (GlyR) are pentameric ligand-gated ion channels that conduct chloride in response to glycine and inhibit neurotransmission in the adult mammalian brainstem and spinal cord. GlyR responses are inhibited by the competitive antagonist strychnine, and taurine is a partial agonist for GlyR (Han et al., 2001; Chepkova et al., 2002). Embryonic GlyR are composed of α_2 subunit homopentamers and are important for synaptogenesis (Kirsch and Betz, 1998). Adult GlyR are heteropentamers, usually composed of three ligand-binding α_1 subunits and two β subunits (Kuhse et al., 1995). Although the α_1 subunit is found primarily in the spinal cord and brainstem, the β subunit is widely distributed in the brain and localizes the GlyR to the postsynaptic membrane by binding to the structural protein gephryn (Malosio et al., 1991; Feng et al., 1998).

Both transgenic and knock-in mouse models provide two approaches for studying the molecular biology of GlyR. Transgenic mice may be generated that express an additional gene (e.g., a GlyR subunit) that may be incorporated into GlyR in the mouse CNS in vivo; however, this approach requires that a promoter (e.g., a tissue-specific promoter) is used to drive expression of the additional gene. The knock-in mouse model provides a way to replace an endogenous gene with another gene (e.g., a gene identical to the endogenous gene except for a point mutation); thus, the knock-in approach utilizes the endogenous promoter to drive expression, which can result in

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doi:10.1016/j.pbb.2005.08.011

an identical pattern of expression. Although the knock-in approach may be in some ways superior, the transgenic approach can provide valuable information, and transgenic mice are typically significantly cheaper and less time consuming to produce. The ways in which these animal models may result in similar and/or differing phenotypes is an ongoing study in science.

Mutations in the GlyR α_1 subunit that decrease GlyR function have been shown to cause human hyperekplexia ("startle disease"), a disease which is characterized by increased acoustic startle responses which can result in uncontrolled falling and apnea (Zhou et al., 2002). Several mouse models of GlyR dysfunction exhibit increased acoustic startle responses (Koch et al., 1996; Kling et al., 1997). Hyperekplexia is characterized by mutations in GlyR and the phenotype can be identified by tapping the bridge of the nose of an infant which results in a startle response and/or hypertonia (Praveen et al., 2001). With hyperekplexia, symptoms usually decrease as the child approaches age 3. Nonetheless, dysfunction or inhibition of receptors other than GlyR (e.g., inhibition of GABA receptors by a cage convulsant) may increase startle responses. For example, a startle phenotype was recently observed in an individual who does not appear to possess any mutations in GlyR (Gaitatzis et al., 2004); however, the phenotype of this individual is distinct from hyperekplexia, and should not be classified as hyperekplexia. Hyperekplexia displays a specific and distinct phenotype and has been observed to be caused by mutations in GlyR.

As evaluated in vitro and in vivo using knock-in mice, the GlyR α_1 S267Q mutation not only eliminates the potentiating effects of ethanol but also decreases the efficacy of glycine by reducing the open channel time, and heterozygous knock-in mice displayed increased startle responses (Findlay et al., 2003). Here we evaluated another mouse model for a similar phenotype as S267Q knock-in mice; mice transgenically expressing the GlyR α_1S267Q mutant were evaluated using several paradigms (acoustic startle, habituation, prepulse inhibition) to determine if transgenic mice display, similar to the knock-in mice, a hyperekplexia-like increase in startle responses and/or alterations in glycine-stimulated strychnine-sensitive chloride uptake. Additionally, spontaneous locomotion was also tested in response to the GlyR partial agonist taurine.

2. Materials and methods

2.1. Generation of mice

Several lines of mice transgenically expressing the GlyR α_1 (S267Q) subunit via the synapsin I neuron-specific promoter were generated as previously described (Findlay et al., 2002). Mice were generated and maintained on an inbred FVB/NJ background, and wild-type littermates were used as control mice in all experiments. Behavioral testing of mice was performed as consistent with the NIH Guide for Care and Use of Laboratory Animals.

2.2. Glycine-stimulated chloride ($^{36}Cl^-$) uptake

Chloride uptake was performed similar to as previously described (Blednov et al., 1996) with modifications. Cortex or brainstem and spinal cord regions were isolated, and the tissue was homogenized in 4.5 ml of ice-cold assay buffer (145 M NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM glucose, 1 mM $CaCl_2$, 10 mM HEPES, pH 7.5 using Tris-base) using a hand homogenizer (Thomas Scientific, Swedesboro, NJ) and then centrifuged at 900 $\times g$ for 15 min and pellets were collected. Cortical microsacs were then resuspended in 8 ml of assay buffer; combined brainstem and spinal cord samples were resuspended in 10 ml assay buffer and then filtered through one layer of 100 μm nylon microfilament cloth (PGC Scientific, Frederick, MD) to remove myelin. Samples were then again centrifuged at 900 $\times g$ for 15 min. The pellet was suspended in ice-cold assay buffer and tissue aliquots (0.8–1.2 μg protein) were incubated at 34 °C for 15 min in the presence or absence of 500 μM strychnine. Strychnine, a specific GlyR antagonist, was included in the buffer in order to block chloride uptake via GlyR, thus providing a way to measure non-GlyR mediated chloride uptake. Strychnine-sensitivity of glycine-stimulated ^{36}Cl uptake (i.e., the ability of strychnine to inhibit glycine-stimulated uptake) was determined by comparing the basal $^{36}Cl^-$ uptake with the glycine-stimulated $^{36}Cl^-$ uptake in the presence of strychnine across multiple experiments. Uptake was initiated by adding 200 μl of $^{36}Cl^-$ solution (2 μCi/ml of assay buffer) containing glycine (50–500 μM final concentration). Nine seconds after addition of $^{36}Cl^-$, influx was terminated by the addition of 4 ml of ice-cold quench buffer (assay buffer containing 0.1 μM strychnine) and rapid filtration using GB100R filters (Advantec MFS Inc., Dublin, CA) and washing once with 8 ml of quench buffer. Filters were incubated in scintillation liquid (4 ml Biosafe II; Research Products International, Mount Prospect, IL) before analysis using a scintillation counter (Beckman LS 6500; Beckman Coulter Inc., Fullerton, CA). The amount of $^{36}Cl^-$ that was bound to the filter in the absence of membrane (no-tissue blank) was subtracted from all values. Glycine-dependent chloride uptake was defined as the amount of $^{36}Cl^-$ taken up while agonist was present in the medium (total intake) minus the amount of $^{36}Cl^-$ taken up when the agonist was not present (glycine-independent or basal uptake). Because both non-radioactive Cl and ^{36}Cl were adsorbed from the assay buffer into tissue samples, calculations have been performed to determine the total amount of chloride taken into the tissue samples, and the total chloride uptake is presented in the figures.

2.3. Acoustic startle response

Acoustic startle responses were measured using SR-LAB test stations and software (San Diego Instruments, San Diego, CA). Test stations were both standardized and calibrated, and startle responses are expressed in mN. In all acoustic startle experiments, transgenic mice from family Tg3 (Findlay et al., 2002) were used and compared to wild-type littermates. Separate mice were used in each test of the acoustic startle

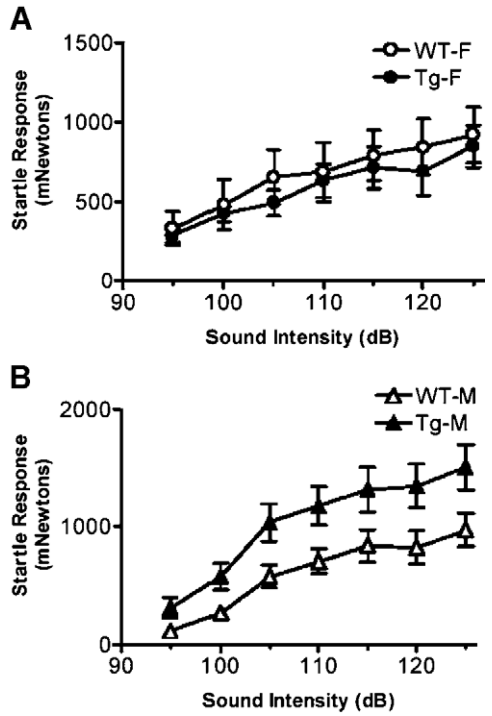

Fig. 1. Acoustic startle responses. A, No differences were observed in the acoustic startle response between transgenic (Tg-F) and wild-type (WT-F) female mice ($n=9$ per group per point). B, The presence of the transgene produced a statistically significant increase in the acoustic startle response of transgenic (Tg-M) male mice compared to wild-type (WT-M) male mice ($p<0.0001$ for the effect of transgene using a two-way ANOVA, $n=14$ per group per point). For both male and female mice the sound intensity produced an increase in startle responses ($p<0.0001$ for both males and females using a two-way ANOVA).

response. Startle responses were recorded as previously described (Bullock et al., 1997). After a 3 min acclimation period, trials of single 40 ms bursts of white noise ranging from 95 to 125 dB were presented in a pseudorandom order with a 10 s inter-trial interval. A background white noise remained at 70 dB for the duration of the experiment. Eight no-stimulus recordings were recorded at pseudorandom intervals to measure spontaneous activity. Each stimulus (with the exception of the no-stimulus trial) was presented 15 times. The entire session comprised 113 trials and took 22 min. The highest and lowest responses to each stimulus intensity and the no-stimulus recordings were excluded. The average response to a stimulus trial at a given sound (dB) intensity was defined as the average startle response minus the average of the background no-stimulus trials.

Habituation to the acoustic startle response was performed as previously described (Ponomarev and Crabbe, 1999). Habituation to the acoustic startle response allows one to obtain a measure of the degree to which a mouse (or other animal) will display a reduced startle response to a repeated loud noise. The noise used in these experiments was a repeated 40 ms pulse of a 120 dB white noise pulse. The 120 dB white noise applied during this short (40 ms) time period was used because this sound is sufficiently loud to elicit a very strong startle response without substantially damaging the hearing of the mouse. The experiments were performed as follows: after a 5 min acclimation period, mice were exposed to a 40 ms burst of white noise (120 dB) above the 70 dB background for a total of 50 trials with an inter-trial interval of 10 s. Data was grouped into 5 blocks of 10 trials for statistical analysis. For each subject the highest and lowest responses for each block of trials were not included.

Prepulse inhibition was determined similar to as previously described (Ralph et al., 2001). First, mice were allowed a 10 min acclimation period. Five 40 ms bursts of white noise (120 dB) were presented at the beginning and the end of the test session. During the test session, six trial types existed: a pulse-alone trial and five prepulse trials; each trial was presented 10 times in a pseudorandom order. The pulse-alone trial was defined as a 40 ms burst of white noise (120 dB). The prepulse trials were defined as a 20 ms prepulse white noise (75–95 dB), a 100 ms pause, and then a 40 ms stimulus noise (120 dB). The inter-trial interval was 10–20 s. The background noise was 70 dB. Ten no-stimulus recordings were interspersed throughout the test session. The "% inhibition" was defined as $=100-[(\text{prepulse response}/\text{pulse alone response})*100]$. In all acoustic startle experiments (acoustic startle, habituation, prepulse inhibition), the strength of the startle response was determined by extracting the maximal level of depression of the startle chamber using a recording of over 65 ms with readings at a 1 ms interval.

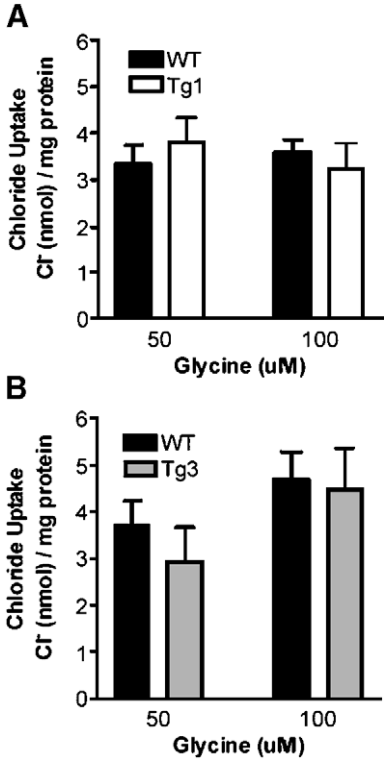

Fig. 2. Glycine-stimulated chloride uptake in synaptoneurosomes from combined spinal cord and brainstem. A, No differences in glycine-stimulated uptake were observed between transgenic (Tg1) and wild-type (WT) mice ($n=8-9$ per group per point). B, No differences in glycine-stimulated chloride uptake were observed in transgenic (Tg3) or wild-type (WT) mice ($n=8-9$ per group per point).

Table 1
Glycine-stimulated $^{36}Cl^-$ flux and ^{3}H-strychnine B_{max} values

	Flux[a]	B_{max}[b]	Flux/B_{max}[c]
Tg3 cortex	7.8±0.9[d]	249±26[d]	32
WT cortex	1.9±0.5	37±10	51
Tg3 spinal[e]	4.5±0.9	599±28	7.5
WT spinale	4.7±0.6	536±22	8.8

[a] Flux is expressed in nmol Cl^-/mg protein.
[b] B_{max} is expressed in fmol ^{3}H-strychnine/mg protein.
[c] Flux/B_{max} is expressed in mol Cl^-/mmol ^{3}H-strychnine.
[d] $p<0.0001$ compared to corresponding wild-type using a two-tailed t-test.
[e] "Spinal" refers to combined spinal cord and brainstem.

2.4. Spontaneous locomotion in response to taurine

Mice were given an intraperitoneal (i.p.) injection of taurine (60 mg/kg) or saline and spontaneous locomotion was measured continuously for three hours as previously described (Findlay et al., 2002). Briefly, locomotor activity was measured using standard mouse cages with Opto-microvarimex (Columbus instruments). Each cage had bedding, food and water, and each cage was covered by a heavy plastic lid with holes for ventilation. Infrared light beams were projected through each cage via the Opto-microvarimex, and locomotor activity was determined by the amount of "light beam breaks" that a mouse would cause as it moves around the cage. Thus a higher number of light beam breaks corresponds with increased locomotion around the cage. The mice were monitored continuously for three hours because locomotion would likely be affected the most by the taurine injection during this time. Both male and female mice were used to test spontaneous locomotion in response to taurine or saline.

2.5. Statistical analysis

Two-way ANOVA, Bonferroni post-tests, and students t-tests were performed where indicated using the Prism 3.0 program (GraphPad Software, San Diego, CA) unless indicated otherwise. All graphs are represented as mean±S.E.M. with levels of significance indicated as *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$.

3. Results

Recently, profound changes in channel function and increases in the acoustic startle responses of mice heterozygous the GlyR α_1S267Q knock-in mutation were observed (Findlay

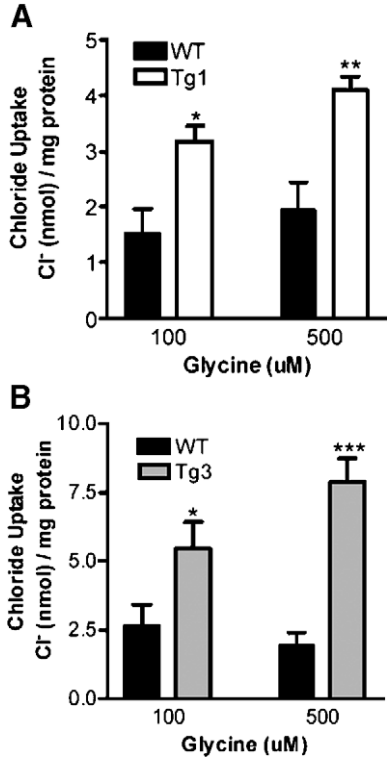

Fig. 3. Glycine-stimulated chloride uptake in cortical microsacs. A, Glycine-stimulated chloride uptake was increased in transgenic (Tg1) mice compared to wild-type littermates. Using a two-way ANOVA, the Tg1 transgene produced a significant increase in the glycine-stimulated chloride uptake ($p<0.0001$). Using Bonferroni post-tests, Tg1 and wild-type mice were significantly different at 100 and 500 μM glycine ($p<0.05$ and $p<0.01$, respectively, $n=6–8$ per group per point). B, Cortical microsacs from transgenic (Tg3) mice displayed greater chloride uptake compared to wild-type (WT) littermates ($p<0.0001$ using a two-way ANOVA). Using Bonferroni post-tests, Tg3 and WT mice were significantly different at 100 and 500 μM glycine ($p<0.05$ and $p<0.001$, respectively, $n=6–8$ per group per point).

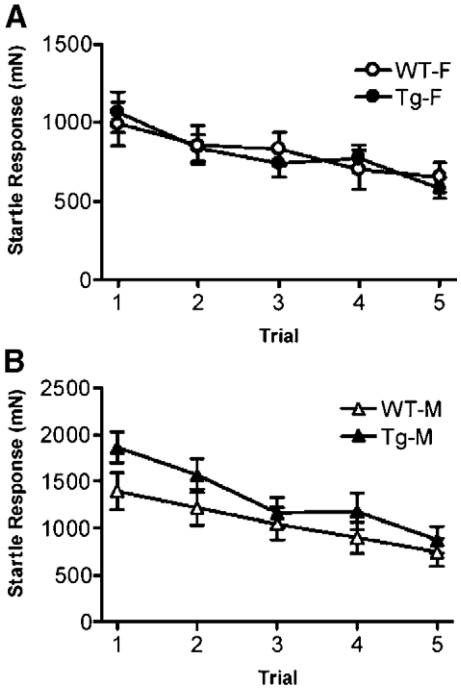

Fig. 4. Habituation to the acoustic startle response. A, No difference in habituation to the startle response was observed for female transgenic (Tg-F) mice compared to wild-type (WT-F) females ($n=14$ per group per point). B, Although transgenic male mice (Tg-M) displayed higher startle responses compared to wild-type males (WT-M) ($p<0.05$ for the effect of transgene using a two-way ANOVA, $n=14$ per group per point), the lack of an (transgene × trial) interaction provides evidence that transgenic and wild-type mice habituate to the startle response at statistically indistinguishable rates. For both male and female mice, subsequent trials produced decreased startle responses ($p<0.01$ and $p<0.0001$ for the effect of trial using a two-way ANOVA for females and males, respectively).

et al., 2003); thus, the hypothesis that mice that transgenically express this S267Q mutation would display a similar phenotype was tested by evaluating the acoustic startle response in transgenic and wild-type mice. In transgenic (Tg3) mice, the presence of the mutant α_1S267Q mutant GlyR subunit resulted in an increase in the acoustic startle response of transgenic male mice (Fig. 1B). However, this result was not observed in transgenic female mice (Fig. 1A). This increase in the startle response was also observed in experiments measuring habituation to the startle response (Fig. 4B); a significant effect of prepulse intensity was observed for both groups of mice ($p<0.0001$, two-way ANOVA).

Because glycine-stimulated chloride flux revealed alterations in GlyR function in knock-in mice harboring the S267Q mutation (Findlay et al., 2003), this technique was used to evaluate GlyR function in mice transgenically expressing the S267Q mutation. Two lines of transgenic mice (Tg1 and Tg3) that express the mutant GlyR α_1 (S267Q) subunit were evaluated and compared to wild-type littermates. Glycine-stimulated chloride uptake using maximal or near-maximal concentrations of glycine was evaluated in brainstem and spinal cord synaptoneurosomes. No differences in chloride uptake were observed as a result of the presence of the transgene in Tg1 or Tg3 mice (Fig. 2). No effect of gender was observed in chloride uptake in cortex or combined spinal cord and brainstem ($p>0.05$). This glycine-stimulated chloride uptake in cortical microsacs and synaptoneurosomes from brainstem and spinal cord was inhibited by strychnine (Fig. 5). By measuring the receptor density and chloride uptake from these tissues, the flux per receptor ratios were estimated (Table 1), and a similar flux per receptor was observed for transgenic and wild-type mice.

Ectopic expression of transgenic GlyR was confirmed using glycine-stimulated chloride uptake in cortical microsacs of transgenic and wild-type mice. Maximal or near-maximal concentrations of glycine were used, and two lines of transgenic mice (Tg1 and Tg3) were evaluated. Cortical microsacs from Tg1 transgenic mice displayed an increase in glycine-stimulated chloride uptake, confirming expression of the transgenic GlyR (Fig. 3A). Glycine-stimulated chloride uptake in cortical microsacs was also increased in Tg3 mice (Fig. 3B). As shown in Table 1, the flux per receptor was about four times higher in tissue from the cortex than brainstem and spinal cord tissue. B_{max} values (Table 1) for ^{3}H-strychnine binding were calculated from ^{3}H-strychnine binding data obtained as previously described (Findlay et al., 2002).

Additional behavioral tests similar to the startle response were also performed. No differences in the rate of habituation were observed as a result of presence of the transgene (Fig. 4). No alterations in prepulse inhibition were observed in male

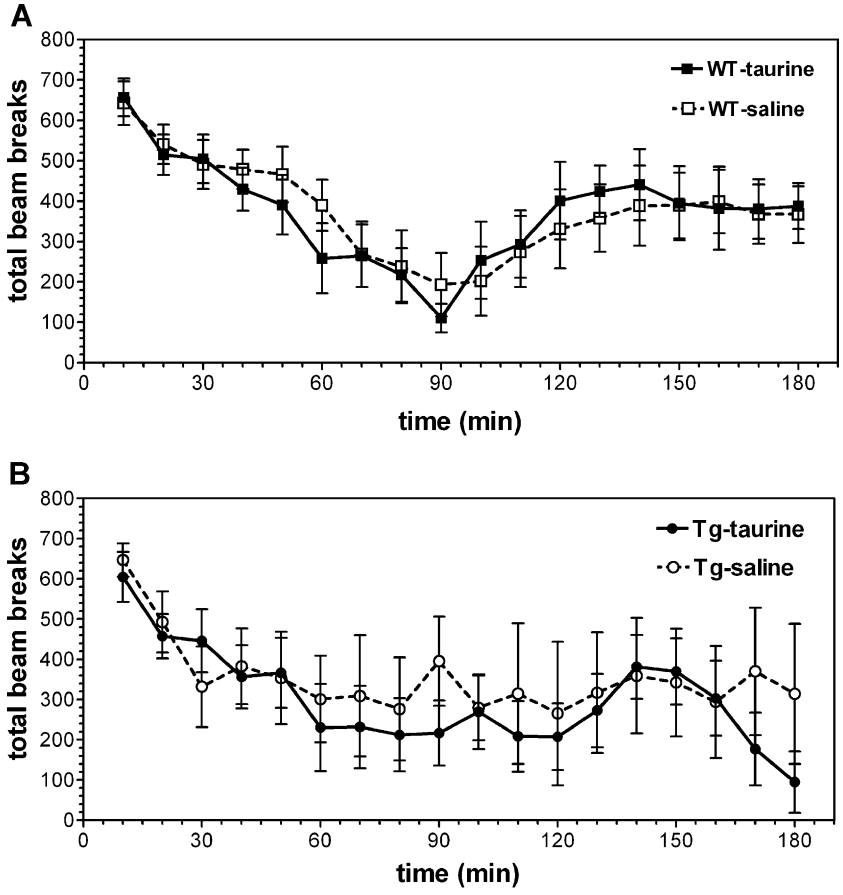

Fig. 5. Spontaneous locomotion in response to taurine or saline. Spontaneous locomotion was tested in transgenic mice injected with taurine (Tg-taurine, 60 mg/kg i.p.) or saline (Tg-saline) and wild type injected with taurine (WT-taurine, 60 mg/kg i.p.) or saline (WT-saline). No effect of transgene or taurine was observed on the spontaneous locomotion ($p>0.05$ for effect of taurine or transgene using two-way ANOVAs, $n=8$ per group).

Tg3 transgenic mice, and no (prepulse × % inhibition) interaction was observed ($p > 0.05$, $n = 14$ per group per point).

The effect of taurine was also evaluated. Although taurine has been shown to alter locomotion using various tests (Sanberg and Ossenkopp, 1977; Ahtee et al., 1985; Aragon et al., 1992), taurine (60 mg/kg) did not alter spontaneous locomotion, and no differences were observed between transgenic and wild-type mice (Fig. 5).

4. Discussion

The observation that GlyR α_1 (S267Q) knock-in mice display a hyperekplexia-like increase in startle responses and decreased glycine-stimulated chloride uptake (Findlay et al., 2003) raises the question as to whether or not transgenic GlyR α_1 (S267Q) mice also display a similar phenotype. We hypothesized that a disruption in GlyR function resulting from transgenic expression of GlyR α_1 (S267Q) would cause a phenotypic increase in acoustic startle responses. Similar to the knock-in mice, male transgenic mice display a hyperekplexia-like increase in startle responses. However, in comparison to the transgenic mice studied here, the knock-in mice displayed dramatic increases in startle responses that were observed for both sexes of mice. Differing genetic backgrounds can affect the magnitude of many behavioral phenotypes (Crawley, 2000), and it is possible that differences in genetic background may be partially responsible or may have influenced some of the differences in the phenotypes. However, the dramatic differences in the severity of the startle phenotype, as compared between transgenic and knock-in mice, support the idea that GlyR function is more disrupted in knock-in, as compared to transgenic, GlyR α_1 (S267Q) mice. It is possible that differing backgrounds may have influenced the observation of a sex difference in the transgenic mice; however, the results suggest that gender differences may be more likely to be observed when a less pronounced disruption in function has occurred (i.e., the transgenic mice as compared to knock-in mice). Additionally, the synapsin I promoter element used to drive expression of the transgenic GlyR carries a steroid binding motif which may contribute to these alterations. Further studies (e.g., sequencing, etc.) may be performed to determine if the synapsin I promoter can influence differences based upon sex, such as the observed sex difference observed herein. The increase in acoustic startle responses in male transgenic mice supports the idea that the transgenic mutant GlyR has disrupted normal GlyR function in the spinal cord and brainstem.

Indeed, many different kinds of disruptions of GlyR function can increase startle responses. For example, specific mutations in GlyR α_1 that decrease GlyR function (e.g., by decreasing glycine potency) can cause dominant hyperekplexia ("startle disease") in humans which is characterized by an exaggerated response to acoustic stimuli which can result in uncontrolled falling (Rees et al., 1994). Heterozygous *Oscillator* (*spd^{ot}*) mice which are heterozygous null mutants for the GlyR α_1 subunit have reductions in GlyR protein and may (Kling et al., 1997), or may not (Findlay et al., 2003), display increased startle responses, and homozygous *spd^{ot}* mice display increased startle responses before they die at approximately two to three weeks after birth (Kling et al., 1997). *Spasmodic* (*spd*) mice carry a GlyR α_1 mutation (A52S) that decreases glycine affinity; these mice also show exaggerated startle responses (Plappert et al., 2001).

The large increases in the acoustic startle response observed in GlyR α_1 (S267Q) knock-in mice (Findlay et al., 2003), as well as the large body of evidence showing the ability of GlyR disruptions to increase startle responses, support the idea that mutant GlyR in the spinal cord and brainstem, and not ectopic S267Q GlyR in the cortex, resulted in the increases in the acoustic startle response of male transgenic mice. Because glycine is not released as a neurotransmitter in the cortex (Gomeza et al., 2003), it is likely that ectopic expression of GlyR in the cortex results in functionally silent receptors. Nonetheless, it is currently not possible to rule out the possibility that ectopic expression of the mutant GlyR α_1 subunit or genetic background could have contributed to increases in startle responses. However, the presence of the transgene did not alter multiple basal behaviors (i.e., in the absence of any drug) as tested previously (Findlay et al., 2002), and it is thus likely that the acoustic startle response is particularly sensitive to changes in GlyR function.

Other behaviors were not altered by the presence of the transgenic S267Q GlyR. The lack of any change in the prepulse inhibition or habituation responses of transgenic mice supports the idea that GlyR modulate the acoustic startle response but not prepulse inhibition or habituation. Prepulse inhibition appears to be a polygenetic trait (Geyer et al., 2002), and there is strong support for the involvement of specific dopamine receptors in prepulse inhibition (Ralph et al., 2001). Additionally, as shown here, spontaneous locomotion in the absence or presence of administered taurine was not altered by the presence of the transgene.

Evidence that the GlyR α_1S267Q disrupts normal GlyR function does not seem to explain the pharmacologically specific decreases in ethanol sensitivity previously observed in the lines of transgenic S267Q mice tested in the present study. Previously, a decreased sensitivity to ethanol was observed in transgenic α_1S267Q mice of both sexes using several behavioral tests (Findlay et al., 2002). Similar to the changes in the acoustic startle response observed here, the ethanol-induced loss of righting reflex response (LORR) was decreased only in male transgenic mice (Findlay et al., 2002). However, because disruptions in GlyR function have been shown to increase LORR duration (Chai, 1961; Kling et al., 1997; Becker et al., 2000; Quinlan et al., 2002), it is unlikely that a disruption in GlyR function would result in a decrease in ethanol-induced LORR duration. Additionally, the loss of righting reflex produced by drugs that alter function of NMDA or $GABA_A$ receptors (i.e. ketamine, flurazepam, pentobarbital) was not altered in transgenic mice (Findlay et al., 2002). These results support the idea that GlyR contribute to some of the acute effects of ethanol. These results are also consistent with the idea that, compared to the acoustic startle response, the LORR behavior may be more resistant to changes as a result of decreases in GlyR function.

The S267Q mutation has been previously shown to disrupt normal GlyR function, and the chloride flux assay was used here to determine if any change in function could be observed in transgenic mice using this technique. Using the *Xenopus* oocyte system, the glycine concentration–response relationship was not altered by the (S267Q) mutation (Findlay et al., 2002); however, further experiments showed a decrease in glycine efficacy as a result of the S267Q mutation (Findlay et al., 2003). Additionally, the channel open time was severely disrupted by the S267Q mutation as shown using single channel recordings (Findlay et al., 2003). Glycine-stimulated strychnine-sensitive chloride flux was evaluated in the present study because it was previously observed that this S267Q mutation reduced chloride uptake in knock-in mice (Findlay et al., 2003).

In contrast to knock-in mice harboring the S267Q mutation (Findlay et al., 2003), no differences in the chloride uptake of spinal cord and brainstem synaptoneurosomes were observed as a result of presence of the transgene. These results are consistent with the less dramatic decrease in the startle response of transgenic mice as compared to knock-in mice (Findlay et al., 2003) and suggest that the S267Q mutation disrupts normal GlyR function in the spinal cord and brainstem to a lesser degree as compared to knock-in mice.

An apparent increase in glycine-stimulated strychnine-sensitive chloride uptake was observed in the cortex of transgenic mice. However, GlyR are normally only expressed in very low levels in the cortex, and the S267Q mutation was previously only shown to decrease, and not eliminate, chloride uptake. Thus, in the present study, the increase in chloride uptake in the cortex of transgenic mice can be interpreted as the observation of ectopic expression of S267Q GlyR in the cortex of transgenic mice. These results confirm earlier observations using ^{3}H-strychnine binding and immunoblotting that showed expression of the mutant transgenic GlyR in the cortex of transgenic mice (Findlay et al., 2002). The results in the present study demonstrate that these ectopic GlyR are able to conduct chloride in response to glycine, but the lack of any decreases in the glycine-stimulated strychnine-sensitive chloride uptake in the spinal cord and brainstem indicates that the chloride uptake assay lacks the sensitivity to detect more subtle changes in channel gating. In contrast, single-channel recordings previously demonstrated a clear disruption in normal GlyR function by the S267Q mutation (Findlay et al., 2003).

Consistent with the small amount of strychnine-sensitive glycine stimulated chloride uptake observed here in the cortex of wild-type mice, previous studies have observed small amounts of GlyR in the cortex. Low levels of the GlyR α_2 subunit are found in layer VI of the adult cortex (Malosio et al., 1991), and these receptors may contribute to the chloride uptake observed here. GlyR responses have also been electrophysiologically recorded in brain regions such as the striatum (Sergeeva and Haas, 2001; Chepkova et al., 2002). It is currently not clear why the chloride flux per B_{max} values are higher in cortical microsacs compared to spinal synaptoneurosomes; however, it is possible that differences in the tissue or method of filtration used on these brain regions may account for these differences observed in transgenic and wild-type mice. Whether the ectopic S267Q GlyR exist in a homomeric (α_1) or heteromeric (α_1:β) state in various regions of the brain might also influence these differences. Expression of the GlyR β subunit is, however, widespread in the brain (Malosio et al., 1991), and, in the mouse model *spastic* (*spa*), reduced levels of the β subunit result in reduced [^{3}H]-strychnine binding (Becker et al., 1992). Increased [^{3}H]-strychnine binding has been previously observed in the cortex of the transgenic mice evaluated here (Findlay et al., 2002), suggesting that the S267Q GlyR exist as heteromeric receptors; however, it is possible that S267Q GlyR may exist as homomers in the cortex, which might influence their function.

In conclusion, similar to knock-in mice, male GlyR α_1 (S267Q) mice display a hyperekplexia-like increase in startle responses. The technique of chloride flux was sufficient to observe ectopic expression of the mutant GlyR but insufficient to observe any disruption of GlyR function as a result of the S267Q mutation. The results here indicate that transgenic mice demonstrate a less dramatic hyperekplexia-like phenotype as compared to knock-in mice and suggest that the acoustic startle response behavior, as compared to other behaviors and chloride flux, is particularly sensitive to disruptions in GlyR function.

Acknowledgements

We would like to thank Virginia Bleck for skillful assistance with genotyping. This work was supported by grants AA0639 and AA13520 from the National Institute of Health.

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