Published in final edited form as:

Brain Res. 2011 January 12; 1368: 290–298. doi:10.1016/j.brainres.2010.10.052.

Profile of Minocycline Neuroprotection in Bilirubin-Induced Auditory System Dysfunction

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23298-0599

Abstract

Excessive hyperbilirubinemia in human neonates can cause permanent dysfunction of the auditory system, as assessed with brainstem auditory evoked potentials (BAEPs). Jaundiced Gunn rat pups (jjs) exhibit similar BAEP abnormalities as hyperbilirubinemic neonates. Sulfadimethoxine (sulfa) administration to jjs, which displaces bilirubin from serum albumin into tissues including brain, exacerbates acute toxicity. Minocycline administered prior to sulfa in jjs protects against BAEP abnormalities. This study evaluates the neuroprotective capabilities of minocycline HCl (50mg/kg) administered 30 or 120min after sulfa (200mg/kg) in 16 day old jjs. BAEPs are recorded at 6 or 24hr post-sulfa. Abnormal BAEP waves exhibit increased latency and decreased amplitude. The sulfa/saline treated jjs exhibited a significantly increased interwave interval between waves I and II (I–II IWI) and significantly decreased amplitudes of waves II and III compared to the saline/saline jjs. The minocycline 30min post-sulfa (sulfa/mino +30) group was not significantly different from the saline/saline control group, indicating neuroprotection. The minocycline 120min post-sulfa (sulfa/mino+120) group had a significantly decreased amplitude of wave III at both 6 and 24hr. These studies indicate that minocycline has a graded neuroprotective effect when administered after acute bilirubin neurotoxicity.

Keywords

Brainstem auditory evoked potentials; neonates; Gunn rats

1. INTRODUCTION

Excessive neonatal jaundice can lead to devastating permanent neurological sequelae involving basal ganglia, auditory system and oculomotor system dysfunction (for review see: Bhutani, et al., 2004; Kaplan and Hammerman, 2004; Larroche, 1968; Shaia, 2005; Shapiro, 2005; Stevenson, et al., 2004; Perlstein, 1960; Volpe, 2001; Maisels, 2000). Bilirubin-induced auditory dysfunction can present as hearing loss due to dysfunction of the auditory nerve (sensorineural) or auditory processing abnormalities due to dysfunction of downstream brain structures (central). Brainstem auditory evoked potentials (BAEPs, or auditory brainstem responses, ABRs), which assess neural transmission between the

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auditory nerve and auditory brainstem structures, can be used to identify sensorineural hearing loss and central auditory processing dysfunction. Basal ganglia dysfunction can present as dystonia (co-contraction of opposing muscle groups) or athetosis (slow writhing movements). If the circulating bilirubin levels can be reduced quickly, permanent brain damage can be avoided. Additionally, there is evidence from case studies (Johnson, et al., 2009) and animal studies (Shapiro, 1993) that with early treatment some neurological dysfunction can be reversed. The American Academy of Pediatrics (2004) recommends phototherapy for term and near-term neonates with bilirubin levels ~15–20mg/dL and exchange transfusion for levels ~20–25mg/dL or greater. Our goal is to identify treatments that can be administered to excessively jaundiced newborns to prevent or attenuate the irreversible brain damage that leads to permanent auditory dysfunction.

Gunn rats have a mutation in an enzyme (UDP- glucuronosyl transferase), which adds glucuronosyl groups to bilirubin rendering it more water soluble and more readily excreted (Johnson et al., 1959; Johnson, et al., 1961; Strebel and Odell, 1971). Homozygous recessive jaundiced animals (jjs) have minimal enzyme activity and exhibit elevated circulating bilirubin levels throughout their lifespan, while heterozygous littermates with half the enzyme activity are non-jaundiced (Nj) (Johnson, et al., 1959). The circulating bilirubin levels peak at 2–3 weeks of age with levels from 8–13 mg/dL, which does not produce any significant impairment in the animals. More consistent neurological sequelae can be produced in 16 day old jaundiced pups by administration of a compound that competes with bilirubin for binding to serum albumin resulting in bilirubin migrating out of the circulation and into lipophilic tissues, including the brain, to produce acute toxicity. Our laboratory typically uses sulfadimethoxine (sulfa) to produce acute bilirubin toxicity as demonstrated with BAEP abnormalities (Shapiro, 1988; Shapiro, 1993; Shapiro, et al., 2007; Geiger, et al., 2007).

Minocycline is a synthetic tetracycline with anti-apoptotic and anti-inflammatory properties that has been shown to be neuroprotective in models of traumatic brain injury (Sanchez-Mejia, et al., 2001; Bye, et al., 2007), stroke (Murata, et al., 2008; Yrjanheikki, et al., 1998; Yrjanheikki, et al., 1999; Carty, et al., 2008), multiple sclerosis (Maier, et al., 2007), Parkinson's Disease (Quintero, et al., 2006), Alzheimer's Disease (Seabrook, et al., 2006) and excitotoxicity (Pi, et al., 2004; Tikka, et al., 2001). Lin, et al (2005) demonstrated that minocycline administered to the dams in the drinking water prevented the cerebellar hypoplasia typically observed in jaundiced Gunn rat pups. This led to our study showing that minocycline administered 15 min prior to sulfa was neuroprotective against bilirubin induced BAEP abnormalities in a dose responsive manner with 50mg/kg being the most effective dose (Geiger et al., 2008). The present study evaluates the neuroprotective effects of 50mg/kg minocycline when administered at 2 different time points after onset of acute bilirubin neurotoxicity.

2. RESULTS

The initial physiological parameters compared between groups were the baseline weight, total plasma bilirubin (TB) and hematocrit (Hct) (Table 1). The ANOVA of these data indicated that there were no significant differences in the baseline values of the physiological parameters between any groups, except the non-jaundiced (Nj) animals uniformly had 0.0mg/dL plasma bilirubin levels, as expected, and thus were significantly lower than all groups of jaundiced (jj) animals ($p < 0.001$).

Animals were treated with sulfadimethoxine (sulfa, 200mg/kg) to induce acute bilirubin toxicity or saline at time zero. Thirty or 120 min after sulfa, minocycline (mino, 50mg/kg) or saline was administered. BAEPs were recorded at 6 or 24hr after sulfa. Representative

BAEP waves of each treatment group are depicted in Figure 1. Figure 1A depicts BAEP recordings at 6hr post-sulfa and Figure 1B depicts BAEP recordings at 24hr post-sulfa in jj animals. The vertical dashed lines provide a visual demonstration of the increased latency of waves II and III following sulfa treatment (acute bilirubin toxicity).

BAEP amplitude and latency values of Nj animals given either sulfa or saline and minocycline 30 min later and saline/saline treated jaundiced animals (jj-sal/sal) were not statistically significantly different from each other at 6 or 24hr, as expected (ANOVA). For statistical comparisons the jj-sal/sal group was used as the control group to which all other groups were compared.

At 6hr, as typically observed between the jj-sulfa/saline (acute bilirubin toxicity) group and the jj-sal/sal control group, the amplitudes of waves II and III and the interwave interval between waves I and II were significantly different ($p=0.023$, $p<0.001$, $p=0.025$, respectively; Figure 2B, D & E), while the amplitude and latency of wave I and the interwave interval between waves II and III (Figure 2A, C & D) were not statistically significantly different. The minocycline treated groups (jj-sulfa/mino+30 and jj-sulfa/mino+120) were not statistically significantly different from the jj-sal/sal with respect to the latency of wave I or the I–II interwave interval. Although the graph of the I–II interwave interval indicates the minocycline treated groups are similar to the jj-sulfa/sal group, the p -values are 0.145 and 0.165 for the mino+30 and mino+120 groups, respectively. The II–III interwave interval was only significantly different from the jj-sal/sal group for the jj-sulfa/mino+120 group ($p=0.043$). The only amplitude in the minocycline treated groups that was significantly different from the jj-sal/sal group was the amplitude of wave III in the jj-sulfa/mino+120 group ($p=0.011$). Thus, at 6 hrs post-sulfa injection the jj-sulfa/sal and the jj-sulfa/mino+120 groups exhibited BAEP abnormalities that were not present in the jj-sal/sal group and the jj-sulfa/mino+30 group was not significantly different from the jj-sal/sal group at any of the BAEP parameters.

Since the time following minocycline until BAEPs was relatively short in the first experiment (only 4hr for the sulfa/mino+120 group) and there might have been abnormalities that would recover over time, we performed a second experiment in which we recorded BAEPs approximately 24hr after sulfa in the same groups of animals. The I–II interwave interval and the amplitudes of waves I, II and III of the jj-sulfa/saline group were significantly different from the jj-sal/sal group ($p=0.013$, 0.002, 0.002, <0.001 , respectively; Figure 3B, D, E & F). This differs from the 6hr time point in that now the amplitude of wave I was significantly reduced compared to the jj-sal/sal control group indicating continued deterioration of the BAEP waveform. The latency parameters (latency of wave I and the I–II and II–III interwave intervals) were not significantly different between the jj-sal/sal group and either minocycline treated group (Figure 3, A, B & C). The amplitudes of waves I, II and III were still not significantly different at 24hr between the jj-sal/sal group and the jj-sulfa/mino+30 group (Figure 3D, E & F). However, the wave III amplitude of the jj-sulfa/mino+120 group had not recovered at 24hr and was still significantly different from the jj-sal/sal group ($p=0.038$) and the wave I amplitude was now significantly reduced ($p=0.01$) indicating deterioration of the BAEP parameters over time similar to the jj-sulfa/sal group.

The jj-sulfa/saline treated group typically lost several grams in the 24hr time period and were frequently administered subcutaneous fluids with dextrose to prevent dehydration ($p<0.001$; Table 2). The weight loss is attributed to the basal ganglia dysfunction induced by bilirubin at this age making nursing difficult to impossible. The sulfa/mino+120 group did not lose weight in this time and was not given fluids, but did not gain as much as the jj-sal/sal control group and was statistically significantly different ($p=0.004$) from the jj-sal/sal group. The sulfa/mino+30 group also did not gain as much as the jj-sal/sal group, but this

difference was not significant. Only the jj-sulfa/saline group exhibited any overt behavioral abnormalities typically observed in this model (data not shown).

3. DISCUSSION

As typically observed between jjs treated with sulfa compared to jjs treated with saline, these studies demonstrate an increase in the I–II interwave interval and decreases in the amplitudes of waves II and III (Shapiro, 1988; Shapiro and Conlee, 1991; Shapiro, 1993; Geiger, et al., 2007; Shapiro, et al., 2008). The jj-sulfa/minocycline+30 group was not significantly different from the jj-saline/saline control group at either 6 or 24 hr indicating neuroprotection at this time point. The jj-sulfa/minocycline+120 group exhibited significant differences in the II–III interwave interval and the wave III amplitude at 6hr, while at 24hr there were no latency differences, but the wave III amplitude decrease persisted and wave I amplitude was now significantly reduced. The jj-sulfa/mino+120 group was not statistically significantly different from the jj-sulfa/mino+30 group at any of the BAEP parameters. These results indicate there is a time-dependent, graded, neuroprotective effect when minocycline is administered after acute bilirubin toxicity, with 30 min being neuroprotective and 120 min being partially neuroprotective.

The jj-sulfa/saline and jj-sulfa/mino+120 groups exhibited a significant decrease of wave I amplitude at 24hr indicating continued deterioration of BAEP waves over time. The jj-sulfa/saline group typically lost weight in 24hr and both minocycline treated groups did not gain as much as the jj-saline/saline group. This weight change indicates insufficient nutritional intake compared to the jj-saline/saline group, which may have contributed to the BAEP abnormalities observed with wave I at 24hr.

Excessive neonatal hyperbilirubinemia is treated with phototherapy and/or blood exchange transfusion (TBs > 15 and 20–25mg/dL, respectively). Exchange transfusion may take hours to arrange after the baby arrives at the hospital and the window of reversibility may have passed by the time the plasma bilirubin level can be sufficiently decreased. Thus, we chose to assess two time points after the onset of acute bilirubin neurotoxicity that may reflect different stages in reversibility or attenuation of BAEP abnormalities. The 30 min post-sulfa injection time for minocycline was chosen based on previous studies as a time when bilirubin was completely displaced from the blood by sulfa in Gunn rats and would represent an initial stage of bilirubin neurotoxicity (Rice and Shapiro, 2006). The 120 min post-sulfa injection time is an early time point when BAEP abnormalities have been observed and reversed with albumin treatment in other studies (Shapiro, 1988; Shapiro, 1993). Human serum albumin injected ip at 2 or 8 hr after sulfa provided recovery of wave II amplitude BAEP abnormalities and partial recovery of the I–II interwave interval at 8–48 hr, although there was not much difference in whether the albumin was injected at 2 or 8 hrs (Shapiro, 1993).

BAEPs are a non-invasive sensitive tool to assess auditory function. BAEP threshold evaluation under various experimental conditions can be used to evaluate ototoxicity of compounds (Husain, et al., 2004; Meli, et al., 2006; Giordano, et al., 2006; Firat, et al., 2008). More information about which region of the peripheral or central nervous system is functioning abnormally can be determined through analysis of the amplitudes and latencies of the waveforms produced. Increased latency of waves indicates increased conduction time and decreased amplitude of waves indicates loss of synchrony of signal.

In bilirubin neurotoxicity, our group assesses latency and amplitude changes of waves I, II and III. In early studies it was determined that wave I is generally not affected by bilirubin, while waves II and III become abnormal displaying increased latencies and decreased

amplitudes (Shapiro, 1988; Shapiro and Hecox, 1988). Wave IV exhibits a decrease in amplitude and actually becomes a lower broader peak, but the latency is not increased (Shapiro, 1988, Shapiro and Conlee, 1991). The decrease in amplitude is likely due to the loss of the input from wave III. Thus, while wave IV may exhibit abnormalities, waves II and III are the most sensitive to bilirubin and our studies focus on those waves. The wave I abnormalities may be nutritionally related as discussed below.

In humans, waves I and II are generated by the auditory nerve, whereas in animals the auditory nerve only produces wave I (Møller, 1994; Markand, 1994). The following wave, III in humans and II in animals is generated by the cochlear nucleus (Møller, 1994; Buchwald and Huang, 1975; Huang, 1980; Fullerton and Kiang, 1990; Zaaroor and Starr, 1991a; Zaaroor and Starr 1991b). There are many bifurcated connections out of the cochlear nucleus that synapse in many other nuclei, some crossing to the contralateral side and others not, so that multiple structures probably contribute to the generation of wave III in rat, although most believe it is primarily from contralateral structures in the superior olivary complex including the lateral lemniscus. Thus, we believe BAEP waves I, II and III in our rat model correspond to wave I–II complex, wave III and wave IV–V complex in humans, and that wave I in rats is from the auditory nerve and wave II is from the cochlear nucleus (Huang, 1980; Buchwald and Huang 1975; Fullerton and Kiang, 1990; Zaaroor and Starr, 1991a; Zaaroor and Starr 1991b). Other studies in rats display waveforms similar to ours and in some the peaks are numbered similarly (Popelar, et al., 2006; Huang, 1980; Galbraith, et al., 2006; Ping, et al., 2007). In our studies the I–II interwave interval is increased and the amplitudes of wave II and III are reduced during acute bilirubin neurotoxicity. Thus, we believe the cochlear nucleus is predominately affected by bilirubin and that the effects on wave III are a consequence of the abnormalities in wave II.

In this study we refined our initial criteria to use animals with plasma bilirubin levels greater than 9.5 to increase the probability that all animals if given sulfa would have abnormal BAEPs and less than 13.5 mg/dL to minimize the mortality. The animals with higher TB levels were not studied, since it would be difficult to obtain sufficient numbers in the jj-sulfa/saline group. However, we expect minocycline would have a graded neuroprotective effect in them also, but perhaps with more BAEP parameters being significantly different from the jj-saline/saline control group. We, also, cannot rule out that the continued deterioration of the BAEP parameters over time in the jj-sulfa/saline group was due to the health status of the animals. It has been shown in other studies that hypoglycemia affects cognitive function and latencies of BAEP waves III and V in humans (Kern, et al., 1994; Münte, et al., 1995; Jacob, et al., 1999; Fruehwald-Schultes, et al., 2000; Strachan, et al., 2003; Høi-Hansen, et al., 2009). It is also known that infants have less glycogen storage reserves than adults and if the pups have not nursed well in 18–24hrs, so that they lost weight, it is possible they could be hypoglycemic, as well as, dehydrated. In the present study, animals that lost weight were given subcutaneous dextrose (5%) in 0.45M sodium chloride, which should attenuate some of the effects of dehydration and of hypoglycemia, if it existed. However, the jj-sulfa/mino+120 group, which gained weight, although not as much as the jj-saline/saline group, also had a decrease in the amplitude of wave I.

In conclusion, we have demonstrated that minocycline administered after the onset of acute bilirubin neurotoxicity has a graded neuroprotective affect in that the sooner after the onset of acute bilirubin toxicity it was administered, the greater the recovery or attenuation of BAEP abnormalities. These studies support the neuroprotective capabilities of minocycline in a neonatal population exposed to excessive plasma bilirubin levels and indicate the sooner it is administered the greater the recovery potential.

4. EXPERIMENTAL PROCEDURE

All procedures were approved by the institutional animal care and use committee of Virginia Commonwealth University and every effort was made to minimize the number of animal used and their pain and distress. This study used 127 Gunn rat pups, 103 jaundiced (jj) and 24 non-jaundiced (Nj) littermates at 16 days of age produced in our Gunn rat breeding colony. Three animals in the 24hr study did not survive. Only litters with at least 3 jjs were used, so each litter had a positive (sulfa treated) and negative (saline treated) control animal. Our preference was for litters with 4 or more jjs so a full complement of jj test groups were included in each litter. Additionally, no more than 2 animals in a group were used from a single litter to minimize skewing of data due to interlitter variability. Initially a blood sample (50–85 μ l) was drawn via a cheek puncture to assess hematocrit and total plasma bilirubin levels (TB) with a Leica Unistat Bilirubinometer (Reichert, Inc., Depew, NY, USA). From unpublished observations we have determined that approximately 50% of jjs with a TB less than 9.0mg/dL exhibit BAEP abnormalities following sulfa treatment, whereas 85% of jjs with TB levels greater than 9.5mg/dL exhibit BAEP abnormalities following sulfa treatment. Animals with higher TB levels (>13.5mg/dL) tended to have higher mortality rates in longer studies. Thus, we refined our experiments to reduce the total numbers of animals required, by only using jjs with TBs between 9.5 and 13.5mg/dL in this study. Animals that lost weight over night were subsequently administered sub-cutaneous fluids (0.45M sodium chloride with 5% dextrose) to attenuate dehydration.

In the first experiment, the animals had blood drawn, were anesthetized, had a baseline BAEP recorded, and then were administered the test compounds. Six hours after the sulfa injection a second BAEP was recorded. Supplemental anesthesia was administered if there was excessive movement artifact. There were no differences in which BAEP parameters were significantly different if we compared differences between baseline and 6hr BAEPs between groups or if we compared just the 6hr data between groups, thus only the 6hr data is presented to facilitate comparison with the 24hr data. In the second experiment, animals had blood drawn and were administered test compounds. Extremely ill animals in the jj-sulfa/saline group were difficult to keep alive for 24hr, so they were administered 5% dextrose in 0.45% NaCl to keep them hydrated. These young animals take hours to wake sufficiently from the anesthesia to nurse adequately and they easily become dehydrated, which can exacerbate the bilirubin neurotoxicity, thus baseline BAEPs were not recorded in the 24hr time-point animals. The test compounds were sulfadimethoxine (200mg/kg, ip) and minocycline HCl (50mg/kg, ip; Sigma Chemical Co.). The vehicle for both was saline (ip). Sulfa (or saline) was administered at time=0. Then, either 30 or 120 min after sulfa, minocycline (or saline) was administered. Thus, the groups were: 1) jjs treated with sulfa followed by saline at 30 min (sulfa/sal - positive control), 2) jjs treated with saline and saline at 30 min (sal/sal – negative control), 3) jjs treated with sulfa followed by minocycline at 30 min (sulfa/mino+30), 4) jjs treated with sulfa followed by minocycline at 120 min (sulfa/mino+120), and 5) Njs treated with sulfa followed by minocycline at 30 min (Nj).

Brainstem auditory evoked potentials (also known as auditory brainstem responses, ABRs) are a very sensitive non-invasive tool to evaluate auditory nerve and brainstem function. Simplistically, they are EEG recordings for the first 10ms following an auditory stimulus (click) averaged following many clicks. As they are averaged the background random EEG becomes neutralized and the waveforms remaining represent the responses of the auditory nerve, the cochlear nucleus and superior olivary complex to the click. Briefly, animals were lightly anesthetized with acepromazine (4.5–6mg/kg) and ketamine (45–60mg/kg) i.m. Supplemental half or quarter doses were administered as needed if muscle artifact became too prominent. BAEPs were recorded using a Nicolet Spirit 2000 Evoked Potential System (Biosys, Inc.). The left ear was occluded with petrolatum, and BAEPs were obtained to

monaural 100 μ second duration rarefaction clicks delivered at 31.7/sec to the right ear through a Sony Walkman 4LIS headphone speaker (Shapiro, et al., 2007; Geiger, et al., 2007; Rice and Shapiro, 2006; Rice and Shapiro 2008). The sound intensity was nominally set at 70 dB, which corresponded to a level of about 62 dB above a normal jj Gunn rat pup BAEP threshold level (Rice and Shapiro, 2006). Surface electrical activity was recorded from 13mm long subcutaneous platinum needle electrodes inserted on the scalp over the vertex and behind the left and right mastoid bullae with a ground electrode in the flank. Rectal temperature was controlled at $37.0 \pm 0.1^\circ\text{C}$ using a controller and heat lamp with a red bulb. The animal's temperature was stabilized for a minimum of 5 minutes before recordings were initiated. Two channel BAEP recordings were obtained from the contralateral to the ipsilateral mastoid (horizontal) and the vertex to the ipsilateral mastoid (vertical) electrode pairs, filtered from 30–3000 Hz. Only the horizontal data is presented. The vertical data is used to help identify uncertain peaks. Each individual BAEP was the averaged response to at least 2000 stimuli, and three or more replicated responses were obtained for each animal. The individual BAEP replications were then added, and the peaks and following troughs were scored using a cursor. The latency of wave I is the time from the stimulus to the peak of wave I. Other stimulus to peak latency values were subtracted to obtain interwave intervals (IWI) between wave peaks to arrive at values for the I–II and II–III interwave intervals. Amplitudes of waves I, II and III are obtained from the peak-to-trough values for each wave. Wave IV is much more variable and historically does not show consistent abnormalities in this model, thus wave IV data is not presented (Shapiro and Hecox 1988 1989).

Physiological data (body weight, total plasma bilirubin level and hematocrit) between the 5 groups (described under Experimental Procedures) were compared by separate one-way ANOVAs with Tukey post-hoc analyses. The BAEP latency data were analyzed with a repeated measures ANOVA to determine if there was a significant main effect. The BAEP amplitude data were also analyzed with a repeated measures ANOVA to determine if there was a significant main effect. For parameters with a significant main effect, one-way ANOVAs were performed to determine group differences at each wave followed by Tukey post-hoc analyses. The p-value was set at $p < 0.05$ for all statistical comparisons. A power analysis determined that with the variability in our BAEP parameters, an $n=10$ has a power of .8049.

Acknowledgments

These studies were supported by NIH R01 grant DC000369 and ADWilliams memorial trust to SMS and Jeffress memorial trust to ACR. We greatly appreciate the assistance of Dr. Robert J. Hamm with the statistical analyses.

Abbreviations

UDP	uridine-di-phosphate
BAEPs	brainstem auditory evoked potentials
ABRs	auditory brainstem responses
Sulfa	sulfadimethoxine
jj	homozygous recessive jaundiced Gunn rats
Nj	heterozygous non-jaundiced Gunn rats

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Figure 1 A.

Figure 1 B.

Figure 1.

Representative BAEP waves at 6 and 24 hr. Representative BAEP waves from each of the jj treated groups: saline/saline, sulfa/mino +30, sulfa/mino +120 and sulfa/saline at A-6hr and B-24hr after sulfa administration. Dashed vertical lines provide visual demonstration of an increasing latency with sulfa treatment in this model. Waves I, II and III are identified. The latency measurements for wave I and the interwave intervals between waves I-II and II-III

are marked as L1, I–II and II–III, respectively. Amplitude measurements are from peak to following trough.

Figure 2. Quantitation of BAEP parameters 6hr after sulfa administration. A- Latency of wave I; B- Interwave interval between waves I–II; C-Interwave interval between waves II–III; D- amplitude of wave I; E-amplitude of wave II; F- amplitude of wave III. Values are mean $\pm$ SE. Significance is at $p < 0.05$. * denotes statistically significantly different from the saline/saline control group.

Figure 3. Quantitation of BAEP parameters 24hr after sulfa administration. A- Latency of wave I; B- Interwave interval between waves I–II; C-Interwave interval between waves II–III; D- amplitude of wave I; E-amplitude of wave II; F- amplitude of wave III. Values are mean $\pm$ SE. Significance is at $p < 0.05$. * denotes statistically significantly different from the saline/saline control group.

Table 1

Physiological Parameters at Baseline (includes both 6hr and 24hr animals).

Group (n)	Wt	TB	Hct
Non-jaundiced (24)	32.2±0.6	0.0±0.0*	34.5±0.5
jj sal/sal (23)	29.7±0.9	11.6±0.2	34.5±0.6
jj sulfa/mino +30 (24)	30.7±0.7	11.6±0.2	33.9±0.6
jj sulfa/mino +120 (21)	31.2±0.7	11.9±0.2	34.9±0.5
jj sulfa/sal (25)	31.2±0.8	10.9±0.2	33.7±0.4

* significantly different from jj sal/sal, p<0.05.

Values are mean ± S.E.

Table 2

Weight change at 24hr.

Group (n)	Weight change (g)
Non-jaundiced (9)	2.24±0.37
jj sal/sal (11)	3.03±0.31
jj sulfa/mino +30 (12)	1.85±0.36
jj sulfa/mino +120 (10)	1.34±0.20*
jj sulfa/sal (13)	-1.97±0.39*

* significantly different from jj sal/sal, $p<0.05$.

Values are mean ± S.E.