

# Acute Brainstem Auditory Evoked Potential Abnormalities in Jaundiced Gunn Rats Given Sulfonamide<sup>1</sup>

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**ABSTRACT.** Bilirubin toxicity produces significant neurologic and audiologic sequelae. Successful therapeutic intervention requires an understanding of the timing of neural dysfunction after exposure to bilirubin. BAEP were used in an animal model of bilirubin encephalopathy to study the onset of neural dysfunction after acute injection of a sulfonamide used to displace bilirubin out of the bloodstream and into tissue. Fourteen pairs of jaundiced Gunn rats from eight litters were studied at postnatal day 18. Baseline BAEP recordings were performed in anesthetized animals; then either sulfadimethoxine or an equal volume of saline was injected into the peritoneum. Another BAEP was done immediately, and then 2, 4, and 8 h after injection. Human serum albumin was injected into an additional 10 animals after the 2-h BAEP recording to see if induced BAEP abnormalities could be corrected. The sulfonamide-treated jj rats developed increased latencies for waves II and III, and I-II and I-III interwave intervals ( $p < 0.0001$ ). The latencies were prolonged by 2 h after injection and became progressively longer at 4 and 8 h. The amplitudes of waves II and III progressively decreased at 2, 4, and 8 h ( $p < 0.0001$ ). Latency and amplitude of waves I and IV did not change. The rats injected with albumin at 2 h showed improvement of BAEP abnormalities at 8 h. These studies show that neurophysiologic abnormalities occur as early as 2 h after intraperitoneal injection of sulfadimethoxine, and are reversible with appropriate therapy. These abnormalities are hypothesized to be due to the sulfonamide driven net transfer of free, toxic bilirubin into the central nervous system. The rapid development of these BAEP abnormalities provides a model system in which the timing and efficacy of therapeutic intervention may be evaluated. (*Pediatr Res* 23: 306-310, 1988)

## Abbreviations

BAEP, brainstem auditory evoked potential(s)  
jj, jaundiced (homozygous)  
Jj, nonjaundiced (heterozygous)  
IWI, interwave interval(s)  
ip, intraperitoneal  
SPL, sound pressure level  
ANOVA, analysis of variance

Bilirubin toxicity produces significant neurologic and audiologic sequelae. Successful therapeutic intervention to prevent such sequelae requires understanding the onset of neural dysfunction after exposure to bilirubin. To better understand the pathogenesis of bilirubin toxicity in the central nervous system we studied acute bilirubin encephalopathy produced by giving an albumin-binding sulfonamide, sulfadimethoxine, to immature jj Gunn rats.

The jj Gunn rat (1), a mutant of the Wistar strain, has an autosomal recessive trait and lacks hepatic glucuronyl transferase activity (2), which converts unconjugated to conjugated bilirubin. Jaundiced Gunn rats have chronic, life-long hyperbilirubinemia which attains peak concentration in the 3rd wk of postnatal life (3, 4). Their neuropathologic abnormalities include damage to central auditory structures, especially the cochlear nuclei and inferior colliculi (5, 6).

The neuropathologic abnormalities are greatly accentuated by giving albumin-binding drugs such as sulfonamide. Sulfonamides displace bilirubin from serum albumin (7); free bilirubin enters brain tissue (8) and produces a pathologic syndrome that closely resembles human kernicterus (3, 4, 9-13). This technique has been widely used in the jj Gunn rat to study the pathogenesis of bilirubin encephalopathy (4, 9, 10).

To document functional abnormalities in acute bilirubin encephalopathy, BAEP were studied in infant jj rats after injecting sulfadimethoxine. After obtaining BAEP abnormalities, human serum albumin was given to decrease brain bilirubin concentration (8) in an attempt to reverse these abnormalities.

## MATERIALS AND METHODS

The recording of BAEP in Gunn rats has been described previously (14). Briefly, animals were anesthetized with ip injection of ketamine (40 mg/kg) and acepromazine (4 mg/kg); smaller supplemental doses were given as needed. Temperature, measured continuously with a deep rectal probe, was maintained at 37.0-37.2° C with a heating pad and a servo-controlled lamp for 10 min before and during BAEP recordings. Subcutaneous platinum electrodes were inserted at the vertex and at the right and left mastoids. Scalp electrical activity was amplified 10<sup>5</sup> (Nicolet HGA-200A), filtered from 30 to 3000 Hz (Nicolet NIC-501A) and averaged (Nicolet 1170) for 10.24 ms poststimulus. The stimuli were 100  $\mu$ s clicks (Grass S88D) at 75 dB SPL delivered at 33.5 clicks/s by a Sony Walkman 4LIS speaker placed over the right external canal just touching the pinna. All recordings were done in a sound attenuated animal chamber (I.A.C. AC-3). Each BAEP, consisting of the averaged response to 2048 clicks, was replicated and plotted separately; for data analysis the two replicated BAEP were added and cursorized digitally according to objective criteria (15).

To assure that sulfadimethoxine injection had the intended

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effect on plasma bilirubin concentration, bilirubin and albumin were measured 4 h after injection of either sulfadimethoxine or saline in pairs of 17-day-old jj rats from four different litters. Bilirubin was measured by the Van den Berg technique (16). Hematofluorometer (AVIV Inc.) automated analyses of total blood bilirubin, albumin bound bilirubin, and saturation index were also measured (17-20).

**Experiment 1.** Fourteen pairs of 18-day-old jj rats from eight litters (maximum of two pairs per litter) were matched by weight and randomly assigned either to the control or experimental group. After baseline BAEP recordings, either sulfadimethoxine 100 mg/kg (Albon, Hoffmann-La Roche Inc.) in a 20% solution with normal saline, or an equal volume of saline was injected into the peritoneal cavity. Another BAEP was done immediately, and BAEP were then recorded at 2, 4, and 8 h after injection. This timing was chosen after preliminary studies established that significant BAEP abnormalities appeared within 15 to 45 min and continued for several more hours after ip sulfadimethoxine injection (Fig. 1).

**Experiment 2.** To establish the potential for reversibility of induced BAEP abnormalities, an additional 10 jj were given sulfadimethoxine 100 mg/kg initially plus human albumin (2 g/kg, ip) 2 h later. Matched littermate controls were given either saline alone or sulfadimethoxine 100 mg/kg, as in the first experiment.

The latencies and amplitudes of waves I, II, III, and IV were chosen by objective criteria (15). In experiment 1 the dependent variables of wave I latency, amplitudes of waves I, II, III, and IV, and the derived measures of the I-II, I-III, I-IV, and II-III IWI (first wave minus second wave; e.g. the I-II interwave interval equals wave II latency minus wave I latency) were examined. In experiment 2 only the measures shown to be of greatest interest in experiment 1 and previous experiments in jj Gunn rats were analyzed: the I-II IWI, and the amplitudes of waves II and III (14, 15).

Statistical analyses were done by mixed design, repeated measures ANOVA (BMDP4V, BMDP Statistical Software). *Post hoc* trend analyses and two-tailed *t* intervals (with correction for unequal variance) were used to show differences between groups.

## RESULTS

BAEP changes in infant jj rats may appear as early as 15 min and are almost always present by 60 min after intraperitoneal

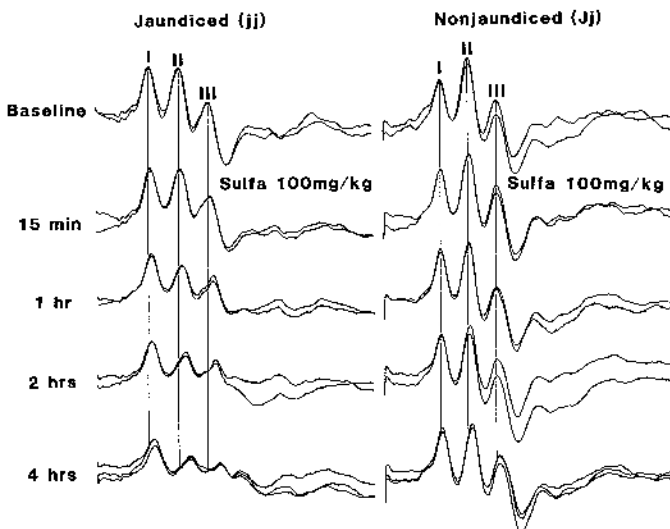


Fig. 1. Sulfadimethoxine injection alters BAEP in infant jj but not Jj. A, BAEP changes are evident within 1 h after sulfadimethoxine in the jaundiced animal. Note the increased IWI of I-II and the decreased amplitudes of waves II and III; I-II is increased by 0.21 ms or 20% by 4 h in the affected jj. B, Jj's BAEP is unaffected by the sulfadimethoxine injection.

injection of sulfadimethoxine (Fig. 1). Sulfadimethoxine injection has no effect on the BAEP of Jj rats (Fig. 1).

Total plasma bilirubin and the molar ratio of bilirubin to albumin decrease in jj rats 4 h after sulfadimethoxine 100 mg/kg ip (Table 1). Hematofluorometer analyses of total blood bilirubin, albumin bound bilirubin, and saturation index (17-20) showed similar results, although this methodology has not been validated in rats.

**Experiment 1.** In the experimental protocol, both groups of jj rats show the expected four BAEP waves pre- and immediately postinjection (Fig. 2). BAEP abnormalities in the sulfonamide-injected jj rats are present in the 2-h recordings and worsen at 4 and 8 h compared to the noninjected control group's BAEP, and

Table 1. Blood and plasma biochemical measurements 4 h after injection of saline ( $n = 4$ ) or 100 mg/kg of sulfadimethoxine ( $n = 4$ ) in 17-day-old jj Gunn rats

	Postsaline		Postsulfa		<i>p</i> *
	Mcan	(SD)	Mean	(SD)	
Total plasma bilirubin (mg/100 ml)	8.0	(1.47)	2.8	(0.54)	0.0007
Bilirubin/albumin molar ratio	0.4	(0.08)	0.1	(0.05)	0.0046
Hematofluorometer					
Total blood bilirubin (mg/100 ml)	11.1	(3.37)	4.9	(0.47)	0.0360
Albumin bound bilirubin (mg/100 ml)	3.5	(0.39)	1.8	(0.22)	0.0015
Saturation index (bound/reserve)	4.5	(0.58)	2.2	(0.50)	0.0020

\* *p* value for the difference between the means by Fischer's exact two-tailed *t* test.

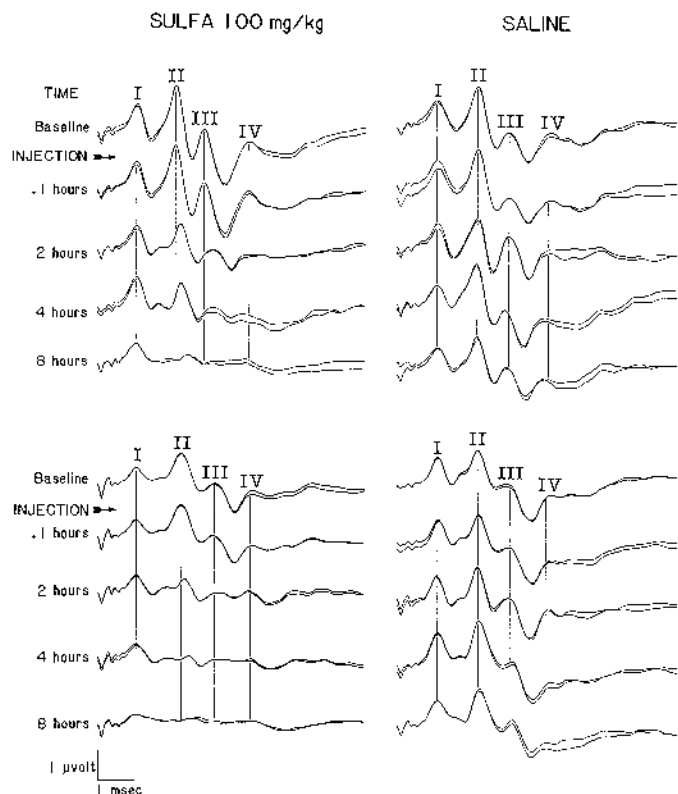


Fig. 2. BAEP recordings from two experimental (left) and two control group animals (right) are shown. The latency and amplitude scales (in ms and  $\mu$ V) are the same for all recordings. Striking changes occur at 2, 4, and 8 h only in the experimental animals. Note the increase in latency and decrease in amplitude of waves II and III. The reproducibility of BAEP in the control animals illustrates the reliability of this technique.

to their own preinjection BAEP. Three of the 14 sulfonamide-injected animals showed some reversal of abnormalities in the 8-h recordings.

Sulfonamide-injected *jj* rats develop increased latencies for the I-II IWI by 2 h postinjection ( $p < 0.0001$ ). The I-II IWI increases by 0.16 ms (15%) over the baseline mean of  $1.11 \pm 0.07$  ms (mean  $\pm$  SD) at 2 h. It progressively lengthens at 4 and 8 h to 0.23 ms (21%) and 0.30 ms (26%) over baseline, respectively (Fig. 3). The I-II IWI also increases in the experimental group compared to the controls at 2 h ( $p = 0.0002$ ), 4 h ( $p < 0.0001$ ), and 8 h ( $p < 0.0001$ ).

The I-III IWI similarly increases over controls ( $p < 0.0001$ ) and over a baseline of  $1.91 \pm 0.11$  ms at 2, 4, and 8 h in the sulfonamide-injected group. However, the increased I-II IWI accounts for most of the increase in I-III. Only at 2 h does the II-III IWI contribute independently to the I-III increase (Fig. 4). The larger variability of II-III in the experimental group at 4 and 8 h is due in part to the difficulty of choosing wave III latency in some animals with abnormal BAEP because wave III amplitude is so small.

Amplitudes of waves II and III progressively decrease at 2, 4, and 8 h (Figs. 5 and 6) only in the sulfadimethoxine injected

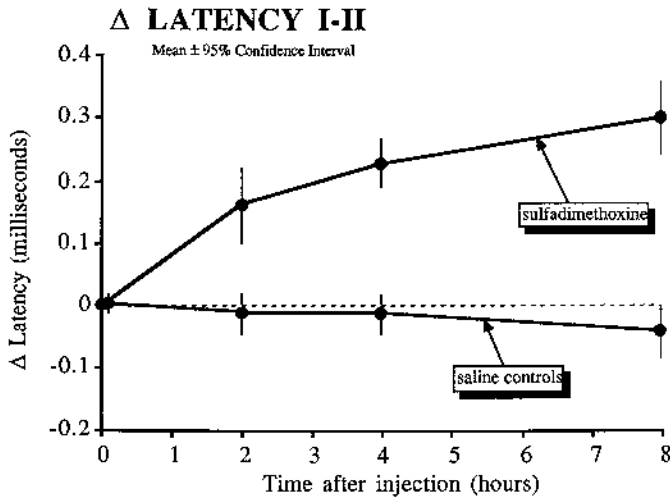


Fig. 3. Change from baseline of IWI I-II in *jj* rats given either sulfadimethoxine or saline. BAEP were done before (time 0), immediately after, and then 2, 4, and 8 h after intraperitoneal injection. The mean latency change from the baseline recordings is plotted in ms; the error bars represent the 95% confidence interval for each mean.

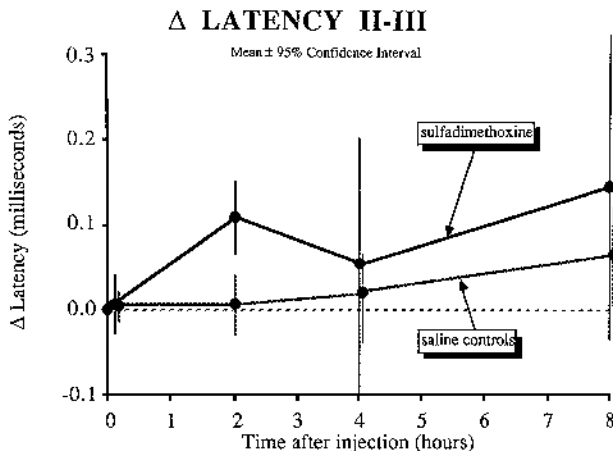


Fig. 4. Change from baseline of IWI II-III in *jj* rats given either sulfadimethoxine or saline (see Fig. 3 for explanation). Note the increase of II-III IWI at 2 and 8 h over baseline, but only at 2 h compared to controls.

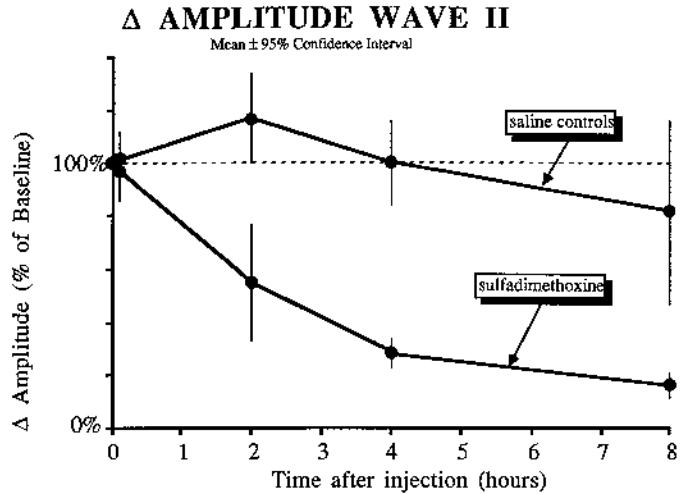


Fig. 5. Amplitude of wave II in *jj* rats given either sulfadimethoxine or saline. The changes in amplitude are expressed as a percentage of the amplitude of the baseline recording; the error bars represent the 95% confidence interval for each mean. Note the highly significant decrease in amplitude of wave II occurring by 2 h after injection of sulfadimethoxine and continuing to decrease at 4 and 8 h.

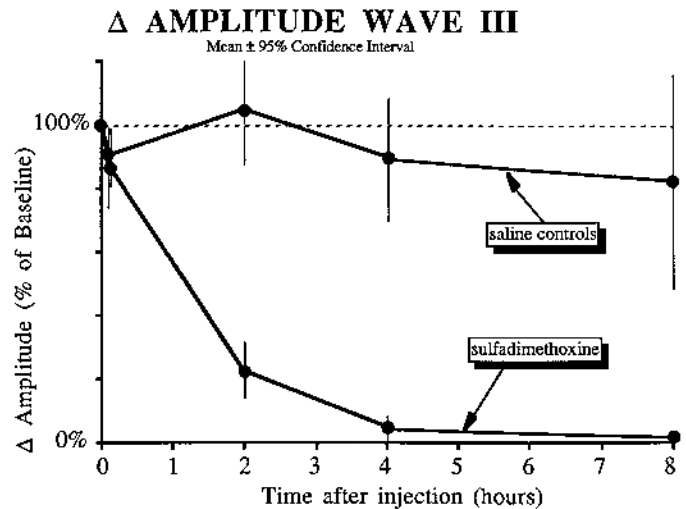


Fig. 6. Amplitude of wave III in *jj* rats given either sulfadimethoxine or saline (see Fig. 5 for explanation). Note the highly significant decrease in amplitude of wave III occurring by 2 h after injection of sulfadimethoxine and continuing to decrease at 4 and 8 h. Wave III had completely disappeared in all but two of the 14 rats at 2 h.

group ( $p < 0.0001$  by repeated measures ANOVAs). Wave II amplitudes are 55, 28, and 16% of baseline of  $1.21 \pm 0.41 \mu\text{V}$ , and wave III is 23, 5, and 2% of baseline of  $0.91 \pm 0.39 \mu\text{V}$  at 2, 4, and 8 h, respectively.

The amplitudes and latencies of waves I and IV, and the I-IV IWI are unaffected after sulfonamide injection. BAEP latencies, IWI, and amplitudes in the saline-injected control rats are initially no different from the sulfadimethoxine-injected group and do not change during the course of the experiment.

*Experiment 2.* The spontaneous recovery of the BAEP abnormalities in some sulfadimethoxine-injected *jj* rats suggests that reversal of neurophysiologic dysfunction is possible. In newborn animals injected with bilirubin, infusion of human serum albumin removes bilirubin previously deposited in brain tissue (8). Human serum albumin, 2 g/kg, was injected into the peritoneum of 10 *jj* rats 2 h after sulfadimethoxine injection.

A partial reversal of the sulfadimethoxine-induced I-II IWI abnormality appears 4 h after the initial sulfadimethoxine injection.

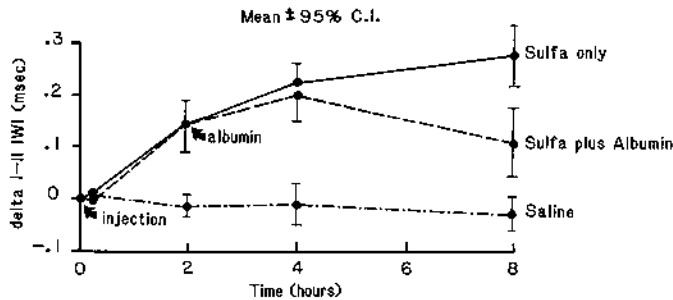


Fig. 7. Reversibility of sulfa-induced I-II IWI abnormality with intraperitoneal albumin. In the sulfa plus albumin group ( $n = 10$ ), albumin 2 mg/kg ip was injected after the 2-h BAEP recording. The sulfa ( $n = 23$ ) and saline ( $n = 19$ ) comparison groups include animals from experiment 1. Note the partial reversal of the abnormal I-II latency beginning at 4 h that becomes statistically significant at 8 h.

tion, *i.e.* 2 h after albumin (Fig. 7). At 8 h the I-II IWI is significantly less than the sulfonamide-injected group not given albumin ( $p = 0.0002$  by one-tailed  $t$  test).

The sulfonamide-induced loss of amplitude of waves II and III is not reversed by albumin by the time of the 4- or 8-h recordings. However, a continued loss of wave II amplitude between 4 and 8 h in the untreated group is prevented by albumin.

#### DISCUSSION

The technique of sulfonamide injection in the *jj* Gunn rat is used to produce histologic abnormalities that are very similar to kernicterus in hyperbilirubinemic human neonates (3, 9–13). It has been argued that the acute bilirubin encephalopathy produced by sulfonamide augmentation mimics human disease better than the chronic bilirubin encephalopathy of *jj* rats not given sulfonamide (10). Findings in the Gunn rat given sulfonamide have in the past had direct impact on clinical practice (21).

The Gunn rat model was chosen because of the considerable information already accumulated, and its clinical, biochemical, and histologic similarities to the human disease state (3, 4, 9). The similarity of Gunn rat BAEP abnormalities to those that occur in hyperbilirubinemic humans (14, 15, 22) further supports this as a good model for studying the neurophysiologic effects of bilirubin encephalopathy. The failure of bilirubin infusion alone to produce BAEP changes in adult normal rats (23) argues against this as a good model, inasmuch as BAEP changes do occur in human bilirubin encephalopathy (24–26).

Results of this study in infant Gunn rats confirm the blood biochemical alterations expected of the sulfonamide injection, namely a lowering of bilirubin and the molar ratio of bilirubin to albumin in the circulation. These are consistent with previous biochemical studies in adult Gunn rats (3, 9, 10, 27), and support the notion that the sulfonamide acts to promote the net transfer of bilirubin out of the circulation and into tissue such as the central nervous system (3, 7–9, 28).

To our knowledge, this is the first study of BAEP in the Gunn rat/sulfonamide model. The acute BAEP abnormalities induced herein are dramatic. Previous studies have found increased I-II IWI and decreased amplitudes of II and III in jaundiced adult (14, 22) and infant (15) Gunn rats. The findings with sulfonamide injection are similar but more severe.

Hyperbilirubinemic human newborns have BAEP abnormalities similar to those in Gunn rats. However, the anatomical localization of the generators of BAEP waves in rats is different than in humans. In humans, wave II arises from the proximal portion of the auditory nerve, outside the brainstem (29), while in rats it is most likely generated in the brainstem cochlear nucleus (30, 31). Wave III in rats has some of the electrophysiologic characteristics of wave V in humans: in vertex to mastoid

differential recordings both are followed by the largest negative trough of the BAEP, and both frequently appear as a smaller wave or plateau riding on the negative deflection of the previous wave (14). Therefore, the increased I-II and I-III IWI herein correspond to the increased I-III and I-V IWI in hyperbilirubinemic human newborns, and the decreased amplitudes of II and III in rats correspond to the decreased amplitudes of III and V in humans (24–26).

The specific changes of Gunn rat BAEP waves suggest the anatomical localization of auditory dysfunction. The I-II IWI and the wave II amplitude abnormalities localize dysfunction to the level of the cochlear nuclei in the brainstem. Abundant histologic evidence in Gunn rats and humans indicates that the cochlear nuclei are extensively damaged by bilirubin (5, 6, 32). Wave III amplitude and latency abnormalities in this study indicate either dysfunction of the ascending auditory pathways rostral to the cochlear nuclei and/or abnormal input from the cochlear nuclei to these pathways. Substituting human waves III and V for rat waves II and III, the same reasoning applies to the localization of human auditory dysfunction with BAEP.

Wave IV, unaffected in this and previous studies of jaundiced rats (14, 15), does not appear to be a sensitive indicator of central auditory abnormalities in acute bilirubin encephalopathy. With serial activation of waves, if waves II and III are altered then wave IV should be altered too. The normal wave IV with abnormal waves II and III suggests that wave IV in the rat may be generated by a pathway parallel to that which generates waves II and III.

The absence of wave I latency and amplitude changes in acute bilirubin encephalopathy suggests that the peripheral auditory system (inner ear and eighth nerve) is not the primary site of auditory pathology in acute bilirubin encephalopathy. This is consistent with morphologic studies that have not demonstrated inner ear abnormalities in Gunn rats (22, 33). However, the possibility that the absence of effect may be due to measurement insensitivity or error cannot be excluded for either wave I or IV.

The BAEP changes appear rapidly. Even though the sulfadimethoxine is injected ip and is a long-acting sulfonamide, abnormalities are often seen in this laboratory 15 min after injection of 100 mg/kg, and almost always by 60 min. This is consistent with the finding by Ahlfors *et al.* (34) of BAEP changes 12 min after a loading intravenous dose of sulfonamide in a premature monkey preinfused with bilirubin. The current study, however, does not focus in detail on BAEP changes occurring less than 2 h after sulfonamide injection. The time course of the earliest BAEP changes would be best studied after intravenous infusion of sulfonamide along with measurement of sulfonamide and bilirubin pharmacokinetics. It is expected that the timing of these early changes relates to 1) sulfadimethoxine dose, route of administration, and absorption rate, 2) the entry rate of bilirubin into the central nervous system, 3) the initial bilirubin concentration, and 4) the availability of bilirubin and sulfadimethoxine binding sites in the circulation.

The BAEP abnormalities are not due to the sulfonamide itself because *Jj* rats injected with sulfonamide do not develop BAEP abnormalities, and sulfonamide alone does not produce histopathology in *Jj* rats (9, 10). The experimental procedures, including anesthesia, are not responsible for the abnormalities because the jaundiced control rats are unaffected.

To our knowledge, this study presents the first evidence of the reversibility of auditory dysfunction due to bilirubin toxicity in a prospectively controlled experiment. Recent studies have shown reversibility of BAEP abnormalities in hyperbilirubinemic human neonates after exchange transfusion (35, 36) and in a premature infant rhesus monkey after albumin after being infused with bilirubin and administered sulfisoxazole (34).

We hypothesize that the neurophysiologic abnormalities occurring earlier than 2 h after intraperitoneal injection of sulfadimethoxine are due to the sulfonamide-driven net transfer of free, toxic bilirubin into the central nervous system, the mechanism

for sulfonamide-induced bilirubin encephalopathy proposed by Odell (7). Measures that promote the net transfer of bilirubin from brain tissue, such as the early administration of human serum albumin, improve neurophysiologic function and may prevent permanent neurologic sequelae.

Brainstem auditory evoked potentials add new dimension to the study of the Gunn rat model of bilirubin encephalopathy. Because BAEP are noninvasive and may be obtained continuously, they may be used to study the time of onset, age dependency, and the reversibility of neurophysiologic dysfunction. The rapid development of these BAEP abnormalities provides a model system in which the timing of therapeutic intervention may be evaluated. In the present study, administration of human serum albumin 2 h after sulfa injection produces a partial reversal of the neurophysiologic dysfunction. Whether reversibility is possible by intervention after longer durations of abnormalities remains to be determined, as do the biochemical and histologic correlates of these functional findings. These experiments dealing with BAEP reversibility in Gunn rats may encourage the development of clinical studies. By using BAEP criteria in addition to clinical and biochemical data, it may be possible to establish better guidelines for the treatment of hyperbilirubinemic neonates.

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