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Short Communication

Reversible blockade of vestibular evoked activity in the chick

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Vestibular evoked potentials (VsEP) were recorded from young chickens following bilateral intralabyrinthine injections of Tetrodotoxin (TTX). The purpose of this study was to document the long term effects of TTX on the electrophysiological activity of the vestibular system. VsEP components were eliminated within 30 min of TTX injections. Twelve hours post-treatment the early waves of the response began to emerge from the background noise. Recordings completed 24 h after the manipulation were not different from baseline responses. Our results indicate that TTX is a useful substance for reversibly blocking vestibular afferent pathways without permanently damaging the labyrinth or neural components. In addition, VsEP is an appropriate tool to objectively evaluate vestibular system function. Their combination can be applied to study the significance of afferent influences on the development and function of vestibular nuclei.

Vestibular evoked potentials; Tetrodotoxin; Avian

Introduction

Manipulations that alter synaptic inputs have been used to study the importance of presynaptic activity on the development and function of the nervous system (Cowan, 1970; Globus, 1975; Rubel and Parks, 1988). Tetrodotoxin (TTX), the active element of the puffer fish poison, has been demonstrated to block nerve conduction without affecting the resting membrane potential (Narahashi et al., 1964). Low concentrations of TTX reversibly block nerve excitation by specifically inhibiting the increase in voltage-dependent sodium conductance of the nerve membrane (Narahashi et al., 1967). This property has made TTX a commonly used drug for inhibiting nerve activity without permanently compromising the subject's anatomy.

Intraocular injections of TTX have been used to study the development of retino-geniculate connectivity in the kitten (Archer et al., 1982). Visual evoked potentials (VEP) were abolished after a single injection of TTX. Similarly, TTX was used to investigate the role of neural activity in the development of cell layers in the dorsal lateral geniculate nucleus (Casagrande and Condo, 1988). VEP were also used to show TTX's effectiveness in action potential blockade. In the auditory system Katsuki et al. (1966), Konishi and Kelsey (1968), Evans and Klinke (1982), Pasic and Rubel (1989), and Born and Rubel (1988) have established that TTX, in the perilymph of mammals and birds, reversibly blocks the activity of the auditory component of the VIIIth cranial nerve.

Vestibular evoked potentials (VsEP) are a new tool for objectively evaluating overall vestibular system integrity (Elidan et al., 1982; Jones and Pedersen, 1989; Weisleder et al., 1989, 1990). For example, this method has been used to examine the effects of aminoglycosides and loop diuretics on the vestibular system (Elidan et al., 1986; 1987). Weisleder et al. (1990) applied the technique to assess the short term influence of unilateral injections of TTX on the vestibular component of the VIIIth cranial nerve.

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The purpose of this investigation was to document the long term effects of TTX on the electrophysiological activity of the vestibular system. VsEP were recorded before and then over a twenty-four hour period following bilateral intralabyrinthine injections of TTX.

Materials and Methods

Eight, two-weak old White Leghorn chicks (*Gallus domesticus*) were tested. Subjects were anesthetized (Ketamine 0.8 mg/10 g body weight and Equithesin 0.015 ml/10 g body weight) and subcutaneous stainless steel electrodes were positioned in a bipolar configuration: vertex (non-inverting input), 2-4 mm behind the opening of the external auditory meatus (inverting input), and skin of the neck (ground). Animals were placed prone on a heating pad with the beak facing down; their temperature was monitored and kept constant at 39°C.

Stimulating and recording methods have been described in detail elsewhere (Jones and Schiltz, 1989; Jones and Pedersen 1989; Weisleder et al., 1989, 1990). In short, an amplified sigmoid-shaped voltage function generated by a computer (PDP 11/73), was applied to a shaker (Ling 203B; solenoid-based linear mechanical vibrator) to produce pulses of linear acceleration. The initial movement of the shaker, in the vertical plane, was an upward displacement. Subjects were tightly coupled to the transducer by embedding the beak, facing down, in a fast-setting plaster pedestal attached to a small Plexiglas platform. This platform was in turn linked to the shaft of the shaker. The animal's head moved in a parallel plane with the beak. An accelerometer (PCB 305A; output 0.5 mV/g where 1.00 $g = 9.81$ m/s^2) continuously monitored movements in the vertical plane. Six acceleration levels, from 0.062 to 2.00 g, were used.

Electrophysiological responses were amplified ($\times$200,000), filtered (100 to 10,000 Hz), and fed to an analog-to-digital converter (12 bit). Each average was the result of 256 samples of the first 10 ms of activity evoked by the stimulus presented at a repetition rate of 6.1/s. The output of the signal averager was displayed on an oscilloscope screen and stored on computer disk for later analysis. All averaged responses were replicated.

After recording baseline responses, bilateral inner ear activity blockade (6 subjects) was achieved through intralabyrinthine injections of TTX (Sigma). Under direct observation (surgical microscope), the tympanic membrane was incised and the columella was removed. The oval window was visualized and a single dose (0.005 ml) of a solution of 2.5×10^{-4} mg of TTX and 12% polyvinyl-alcohol (PVA; DuPont) was injected. Two additional subjects received injections in which only PVA was administered. Electrophysiological activity in response to linear acceleration was recorded 0.5, 3, 6, 12, and 24 h following these manipulations. Between recording sessions anesthesia was discontinued. Animals were reanesthetized before each recording session by providing supplemental dosages of the anesthetics.

Results

Fig. 1A presents a representative time series recorded from a subject before and after TTX injections (acceleration level 2.00 g). A response recorded from a different subject six hours after a PVA-only injection is displayed in Fig. 1B. In all subjects TTX injections in PVA eliminated all VsEP components within 30 min of the injection. We were also unable to identify any of the characteristic waves in recordings completed 3 and 6 h after the TTX injections. Twelve hours post-injection the early components of the VsEP (P-1, N-1, and P-2) began to emerge from the background noise. Twenty four hours after the manipulation we recorded responses in which the morphology did not differ from that obtained before the injections. The traces presented in Fig. 1B demonstrate that injections of the embedding solution do not alter the morphology of the response (compare to intact response on panel A in same figure).

Threshold was defined as the level in between the last acceleration at which P-2, the most prominent component of the VsEP, was recognized and the acceleration at which P-2 could not be identified. Mean threshold for intact animals was 0.07 g [standard error of mean (SEM) $\pm$ 0.01]. Average response threshold twelve hours after an injection of TTX in PVA was 0.27 g. Threshold values

returned to within normal limits (mean 0.1 g; SEM $\pm$ 0.01) by 24 h post-injection (see Fig. 2). One factor repeated measures analysis of variance (ANOVA) revealed a reliable effect of treatments on the response threshold [F (2, 10) = 6.92, $P <$ 0.01]. Post-hoc pairwise comparisons (Fisher's Least Significant Differences) showed reliable differences between the thresholds from intact animals and those from the same animals twelve hours after the TTX injection ($P < 0.05$). Similarly, thresholds at twelve hours were significantly higher from those recorded at twenty four hours ($P < 0.05$). No significant difference in threshold was found between the intact condition and twenty four hours after TTX injections. Mean threshold response for the two PVA-only subjects (0.07 g), recorded six hours after the injection, was not different from that of intact subjects.

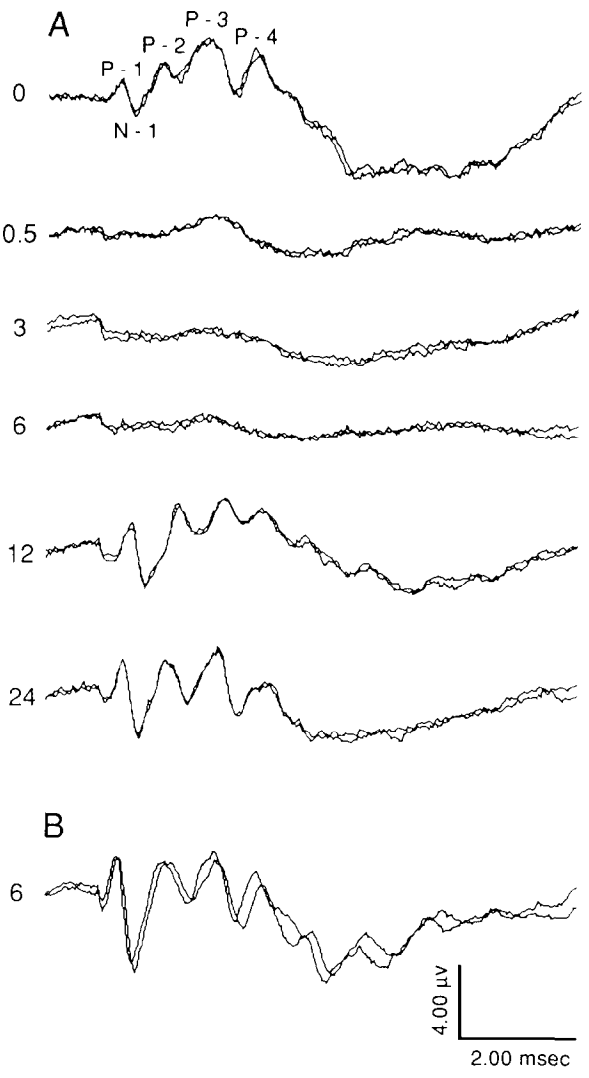

Fig. 1. (A) VsEPs recorded before and over a twenty-four hour period following bilateral intralabyrinthine injections of TTX. Number to the left of trace indicates hours after injection. First trace displays VsEP nomenclature. (B) Response obtained from a subject six hours after bilateral injections of the embedding vehicle only. Use initial recording (0) from experimental subject in A for comparison. All averages are replicated to show consistency.

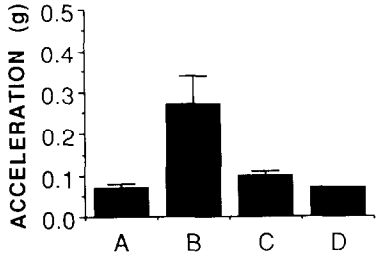

Fig. 2. Mean threshold level of responses recorded from subjects at three different times: (A) pre-injection responses, (B) twelve, and (C) twenty-four hours after TTX injections. Also, (D) mean threshold at six hours from subjects that only received injections of embedding vehicle. (Error bars = 1 SEM).

The average wave P-2 latency for intact animals at 2.00 g was 2.28 ms. Twelve hours after the injection mean P-2 latency was 2.99 ms. The latency of P-2 returned to within normal limits by twenty four hours (2.38 ms) (See Fig. 3). One factor repeated measures ANOVA revealed a reliable effect of treatments on the latency [F (2, 10) = 11.4, $P < 0.01$]. Post-hoc pairwise comparisons (Fisher's Least Significant Differences) showed reliable differences between the intact and twelve hours ($P < 0.01$) as well as between twelve and twenty four hours ($P < 0.01$). Significant differences in P-2 latency were not found between the intact condition and the twenty four hour condition. Mean latency of wave P-2 in PVA-only subjects (2.25) was not reliably different from that of intact animals.

In summary, the statistical analyses confirmed that threshold and latency of wave P-2, measured twelve hours after bilateral injections of TTX, are different from those recorded prior to the manipulation. Response parameters returned to within normal limits by twenty four hours after the injec-

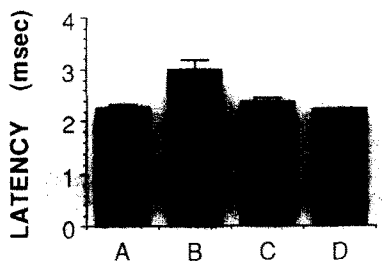

Fig. 3. Mean wave P-2 latency recorded from subjects at three different times: (A) pre-injection responses, (B) twelve, and (C) twenty-four hours after TTX injections. Also (D) mean latency at six hours from subjects that only received injections of the embedding vehicle. (Error bars = 1 SEM).

tion. Injections of the embedding vehicle without TTX did not alter the morphology, threshold, or latency of the VsEP.

Discussion

The objective of this study was to characterize the long term effects of TTX on vestibular evoked responses elicited by linear acceleration. Bilateral intralabyrinthine injections of TTX abolished VsEP components within a half hour. Characteristic waves could not be recognized 3 or 6 h after the treatment. Recordings completed at 12 h revealed prolonged wave P-2 latency and threshold elevation. VsEPs returned to within normal limits 24 h after the injection, although the actual threshold and latency values appeared slightly elevated.

The results from our experiments show that small amounts of TTX reversibly block the afferent pathways of the vestibular system. Previous investigations enable us to rule out any cochlear-transduced contributions to the electrophysiological activity in response to acceleration stimuli. Jones and Pedersen (1989), and Weisleder et al. (1989) have shown that high levels of acoustic white noise do not affect the VsEP. Furthermore, Weisleder et al. (1990) successfully recorded VsEP following bilateral cochlea removal. Thus, the changes in response latency and threshold reported in the present paper do not reflect the effects of TTX on the auditory system.

The short-term effect that bilateral injections of TTX have on the electrophysiological activity in response to linear acceleration stimuli (see also Weisleder et al., 1990) indicate that the response originates within the inner ear. Disappearance of VsEP's characteristic waves also rules out electrode motion artifact and somatosensory activity as the source of these potentials.

TTX has been used to study the importance of afferent activity on the development and function of several sensory systems. Archer et al. (1982) reported abnormal development of retino-geniculate synaptic connections in kittens who received intraocular injections of TTX. Significant reduction of total dendritic spines in developing-rat visual cortex was reported by Riccio and Matthews (1985) following intraocular TTX. In the auditory system, Born and Rubel (1988) and Pasic and Rubel (1989) reported decreases in protein synthesis and auditory nuclei cell size following TTX injections. Finally, protein synthesis in nucleus vestibularis tangentialis was found to be reduced following intralabyrinthine TTX injections (Bohrer et al., 1988). The results from our investigation support the notion that TTX is a dependable blocker of vestibular afferent activity. Additionally, we have provided evidence that VsEPs are a reliable method of monitoring the effects of TTX on the vestibular system. Used in conjunction, these two tools can be applied to study the significance of presynaptic inputs on the development and function of vestibular areas of the brain.

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References

Archer, S.M., Dubin, M.W. and Stark, L.A. (1982) Abnormal development of kitten retino-geniculate connectivity in the absence of action potentials. Science 219, 743–745.

Bohrer, P.S., Born, D.E. and Hyson, R.L. (1988) Activity-related regulation of protein synthesis in brain stem vestibular nuclei of the chicken. Abstracts of the Association for Research in Otolaryngology, pp. 58–59.

Born, D.E. and Rubel, E.W (1988) Afferent influences on brain stem auditory nuclei of the chicken: Presynaptic action potentials regulate protein synthesis in Nucleus Magnocellularis neurons. J. Neurosci. 8, 901–919.

Casagrande, V.A. and Condo, G.J. (1988) The effect of altered neuronal activity on the development of layers in the lateral geniculate nucleus. J. Neurosci. 8, 395–416.

Cowan, W.M. (1970) Anterograde and retrograde transneuronal degeneration in the central and peripheral nervous system. In: W.J.H. Nauta and S.O.E. Ebbesson (Eds.), Contemporary Research Methods in Neuroanatomy, Springer-Verlag, New York, pp. 217–251.

Elidan, J., Sohmer, H. and Nitzan, M. (1982) Recording of short latency vestibular evoked potentials to acceleration in rats by means of skin electrodes. Electroenceph. Clin. Neurophysiol. 53, 501–505.

Elidan, J., Sohmer, H. and Lev, S. (1984) Short latency vestibular evoked responses to acceleration stimuli recorded by skin electrodes. Ann. Otol. Rhynol. Laryngol. 93, 257–261.

Elidan, J., Lin, J. and Honrubia, V. (1986) The effect of loop diuretics on the vestibular system. Assessment by recording the vestibular evoked response. Arch. Otolaryngol. 112, 836–839.

Elidan, J., Lin, J. and Honrubia, V. (1987) Vestibular ototoxicity of gentamicin assessed by the recording of a short-latency vestibular evoked response in cats. Laryngoscope 97, 865–870.

Evans, E.F. and Klinke, R. (1982) The effects of intracochlear cyanide and tetrodotoxin on the properties of single cochlear nerve fibers in the cat. J. Physiol. 331, 385–408.

Globus, A. (1975) Brain morphology as a function of presynaptic morphology and activity. In: A.H. Riesen (Ed.), Developmental Neuropsychology of Sensory Deprivation, Clarendon Press, Oxford, UK, pp. 299–326.

Jones, T.A. and Schiltz, T.J. (1989) Pulsed linear acceleration as a vestibular stimulus in electrophysiological investigations. J. Neurosc. Methods 27, 115–120.

Jones, T.A. and Pedersen, T.L. (1989) Short latency vestibular responses to pulsed linear acceleration. Am. J. Otolaryngol. 10, 327–335.

Katsuki, Y., Yanagisawa, K. and Kanzaki, J. (1966) Tetraethylammonium and tetrodotoxin: Effects on cochlear potentials. Science 151, 1544–1545.

Konoshi, T. and Kelsey, E. (1968) Effect of tetrodotoxin and procaine on cochlear potentials. J. Acoust. Soc. Am. 43, 471–480.

Narahashi, T., Moore, J.W. and Scott, W.R. (1964) Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. J. Gen. Physiol. 47, 965–974.

Narahashi, T., Haas, H.G. and Therrien, E.F. (1967) Saxitoxin and Tetrodotoxin: Comparison of nerve blocking mechanism. Science 147, 1441–1442.

Pasic, T.R. and Rubel, E.W (1989) Rapid changes in cochlear nucleus cell size following blockade of auditory nerve electrical activity in gerbils. J. Comp. Neurol. 283, 474–480.

Riccio, R.V. and Matthews, M.A. (1985) Effects of intraocular Tetrodotoxin on dendritic spines in the developing rat visual cortex: A Golgi analysis. Exp Brain Res; 19: 173–182.

Rubel, E.W and Parks, T. (1988) The developing auditory system. In: G.M. Edelman, W.E. Gall, and W.M. Cowan (Eds.), Auditory Function, John Wiley and Sons, Inc., pp. 3 92.

Weisleder, P., Jones, T.A. and Rubel, E.W (1989) Potenciales evocados vestibulares en *Gallus domesticus*. Archivos de Neurobiologia. (Spain). 52. 307–313.

Weisleder, P., Jones, T.A. and Rubel, E.W (1990) Peripheral generators of the vestibular evoked potentials in the chick. Electroenceph. Clin. Neurophysiol. (in press).