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TETRODOTOXIN AND THE ELECTROCORTICAL RESPONSE TO LIGHT

BY

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The effect of tetrodotoxin upon the central nervous system has been considered by Rech, McCarthy & Borison (1964) and more recently by Frank & Pinsky (1966) with some inconsistency in results between the two groups, in that the former found no significant changes in the electroencephalogram, while the latter reported inhibition of potentials evoked by electrical stimulation in isolated cortical slabs. The present study measured the change in photic evoked potentials and electroencephalograms in rat following injection of tetrodotoxin into the lateral cerebral ventricle.

METHODS

Thirty albino rats (250–350 g and 90–150 days old) were immobilized by succinylcholine, artificially ventilated and held in a sterotaxic instrument. Lignocaine hydrochloride was injected at points of incision and eye dilatation was fixed by atropine sulphate. The succinylcholine was given intraperitoneally in sufficient amount to inhibit withdrawal of the hind foot to a pinch with forceps, while a drop of atropine was placed in each eye at the beginning of the experiment and no stimulations were initiated until complete dilatation was observed; no additional doses of atropine were required for the duration of the experiment. Therefore, succinylcholine and atropine conditions were comparable over control and experimental conditions. Photic stimulation was from a Grass PS-2 stimulator lamp 12 cm in front of the subject. A hole 5 mm in diameter was trephined over the occipito-parietal area, care being taken not to damage the dura. A bi-polar electrode was placed on the dura and covered with a drop of mineral oil. The photic evoked potentials were amplified by a Grass P511 preamplifier, monitored on an oscilloscope, and summed on a CAT computer. The electroencephalogram was continuously recorded by a P-7 polygraph. A level of photic stimulation was selected which produced on observable photic evoked potential approximately half the time. Responses to fifty stimulations were summed before and after control and experimental injections. The rate of stimulation was variable at about 1/sec. All stimulations were done under dim room lighting and after a 15–25 min adaptation period.

Tetrodotoxin (Kao, 1966), control, and reference drug solutions were introduced in comparable volumes (6 μl.) by microsyringe. Solutions prepared using the crystal powder and carbonic acid solution forms of tetrodotoxin (Sankyo, Japan) were found to be comparable in potency. Intracerebroventricular injection was into the lateral ventricle just lateral to the septum and ventral to the corpus callosum. Histological examination verified the cannula placement. When sub-dural injections were given, the dura puncture was lateral to the electrodes allowing the solution to flow over the cortex and between the electrodes which were separated by a distance of 2 mm. With intra-

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peritoneal injections, the syringe and needle were put in place at the beginning and remained in place throughout the experimental period. As the needle was inserted in tissue at all times, the subsequent micro-injections were at body temperature. Control solutions were distilled water or saline, while reference drugs were cocaine hydrochloride and tubocurarine—all given in equal volume. No changes were ever observed following control solution injections.

RESULTS

Tetrodotoxin (28 μg and 6 μl.) always produced a complete inhibition of the photic evoked potentials and produced significant changes in the electroencephalogram. Figure 1 demonstrates the maximum effect upon photic evoked potentials and electroencephalo-

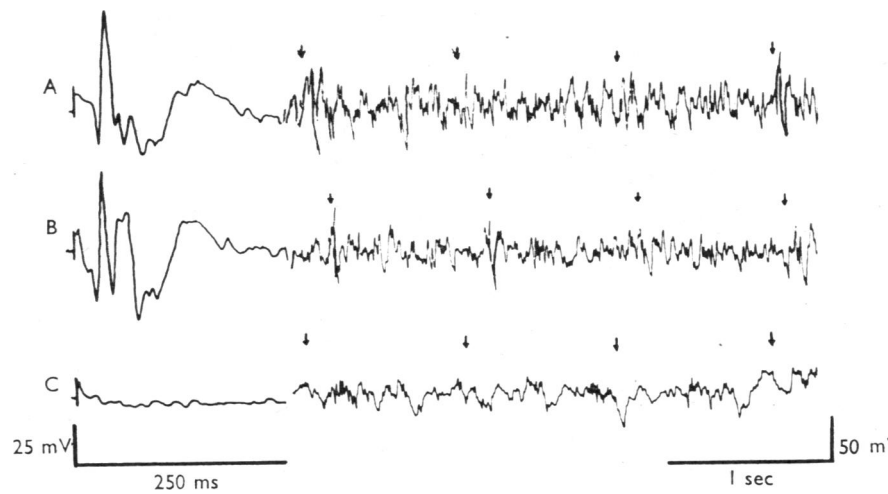

Fig. 1. Photic evoked potentials: A=control; B=90 min after cocaine HCl (6 μl. and 19 μg.); and C=5 min after tetrodotoxin (6 μl. and 28 μg).

grams by cocaine hydrochloride (19 μg and 6 μl.) as compared with subsequent injection of tetrodotoxin. Photic evoked potentials and electroencephalogram samples taken over the 90 min period following cocaine presented a consistent pattern of positive-negative waveform of photic evoked potentials with clear spike-and-spindle in the electroencephalograms. Within 5 min of the injection of tetrodotoxin there was no potential evoked by stimulation and the electroencephalogram pattern had changed to a high and low frequency combination with reduced amplitude. The stability of the photic evoked potentials and electroencephalograms after tubocurarine (18 μg in 6 μl.) is shown in Fig. 2, even though the tubocurarine induced an electrical seizure of 3 min duration at 6 min after the injection. It is seen that the photic evoked potentials and spike-and-spindle electroencephalogram pattern remained consistent. However, after 5 min, tetrodotoxin reduced the photic evoked potentials amplitude and altered the electroencephalogram pattern, reaching the maximal effect by 10 min post-injection. Local stimulation of the cortex by cleaning the dura is reflected in Fig. 2 F as the electroencephalogram increased in amplitude and complexity while the photic evoked potential was not in evidence.

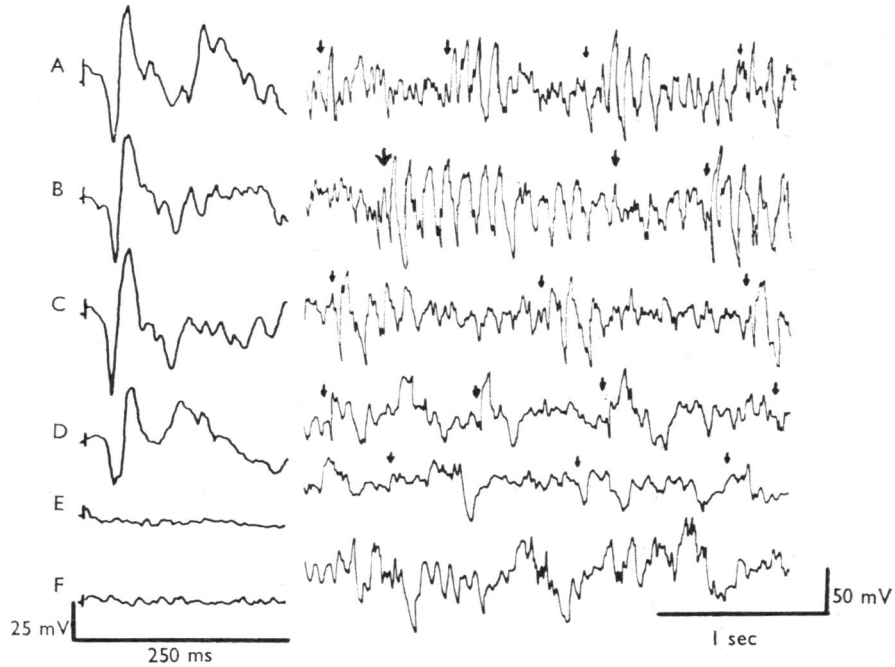

Fig. 2. Averaged curves of fifty photic stimulations under conditions: (A) control; (B) 5 min after 6 μl. and 18 μg tubocurarine; (C) 30 min after (B) and pre-tetrodotoxin; (D) 5 min after 28 μg tetrodotoxin in 6 μl. to lateral ventricle; (E) 10 min after tetrodotoxin; and (F) electrodes raised, cleaned, and replaced. Analysis time 250 ms.

DISCUSSION

Intraperitoneal injections of tetrodotoxin in ten subjects never produced an observable change in the photic evoked potentials wave-form or amplitude. This held true when the dose was increased more than five-fold, or to 150 μg. Since the normal lethal effect is by blockade of the myoneural junction resulting in respiratory arrest, this effect was not observable in the artificially ventilated preparation. The absence of an effect upon the electrical pattern or responsiveness of the central visual system may be due to the binding of tetrodotoxin at myoneural junctions before reaching the central nervous system, or exclusion from the central nervous system by a blood-cerebrospinal fluid barrier mechanism. Evaluation of these possibilities are planned.

SUMMARY

Tetrodotoxin depresses the central nervous system visual system when deposited in the cerebrospinal fluid (lateral ventricle). The effect was not obtained with intraperitoneal injections even at massive doses.

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