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Effect of Staphylococcal Enterotoxin B
on the Electroencephalogram of Monkeys

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A highly purified preparation of staphylococcal enterotoxin B was administered intravenously, 1 mg/kg, to rhesus monkeys. Electroencephalograms (EEG) were recorded from electrodes attached to the skin or implanted on the dura. The dose of toxin employed consistently produced a sequence of vascular collapse followed by death; in control studies, animals were bled periodically to produce a similar pattern of shock. Regardless of the time to death following administration of the enterotoxin, there were essentially no changes from base line EEG patterns until shortly before death. With the development of preterminal severe shock, there was a marked decrease in EEG wave frequency and an initial increase in amplitude. The latter diminished progressively to produce an isoelectric tracing immediately prior to death. This could be reversed for a brief period by epinephrine. An identical sequence of EEG changes was observed during the terminal period of hemorrhagic shock. It is postulated that cerebral anoxia, caused by inadequate blood flow, is the primary cause of the altered EEG patterns that accompany enterotoxin toxicity. In this respect, staphylococcal enterotoxin B produces changes apparently similar to bacterial endotoxin but distinctly different from the EEG effects reported after botulinum toxin, anthrax toxin, or rattlesnake and cobra venom.

Staphylococcal enterotoxin B (SEB) is a simple protein exotoxin which, following large intravenous (iv) doses to monkeys, produces fever, anorexia, emesis, diarrhea, oliguria, severe depression, terminal shock, and death (4, 10).

The effects of SEB on the central nervous systems are not well documented. Sugiyama (15) has stated that the rhesus monkey is completely refractory to the emetic effect of partially purified staphylococcal enterotoxin administered orally after bilateral destruction of the area postrema on the floor of the fourth ventricle. Dirks (*personal communication*), utilizing the fluorescent-antibody technique, has detected SEB within neurons of brain sections taken from monkeys sacrificed 4 to 24 hr after iv challenge.

The electrical activity of the cerebral cortex of monkeys is altered by various lethal toxins of biological origin (5, 9, 14, 16). Type A botulinum toxin (9) and crude anthrax toxin (5) both produce changes in electrical activity of the cerebral cortex, consisting of cycling among an electrically silent electroencephalogram (EEG), a period of

increased amplitude in electrical impulses, and an apparently normal EEG. Crude venom of the cobra (*Naja naja*) and rattlesnake (*Crotalus admonteus*) (16) produce alterations in the EEG of dogs and monkeys, characterized by a complete loss of electrical activity within 1 min after iv administration. *Escherichia coli* endotoxin, which produced a clinical syndrome quite similar to that produced by SEB in monkeys (14), apparently has no effect on the EEG per se, although in the late stages of endotoxin shock EEG alterations attributed to progressive anoxia are noted (16).

Because of these differences in central nervous system responses to various biological toxins, we have recorded and analyzed the EEG patterns of monkeys after SEB challenge, and have compared the changes with those previously described after challenge with other toxins.

MATERIALS AND METHODS

The SEB employed in these studies was a highly purified preparation, extensively characterized by Schantz et al. (12), and used in this laboratory in various physiological studies (4, 10).

Twelve monkeys (*Macaca mulatta*), weighing 2.3 to 3.0 kg, were utilized in this study. The first group of four monkeys was anesthetized with sodium pentothal through an indwelling saphenous vein catheter

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and the monkeys were held at the stage of light surgical anesthesia Three surface electrodes were attached to the shaven skin of the left and right occipital and the right frontal regions. Control tracings were obtained immediately thereafter Three were challenged iv with 1,000 μg of SEB per kg of body weight, a dose generally lethal, and the fourth was bled periodically to determine the effects of hemorrhagic shock.

In the second group, consisting of eight monkeys, two silver electrodes were implanted directly on the dura of each hemisphere by standard sterotaxic techniques a minimum of 2 days prior to challenge or bleeding. The EEG was recorded in the nonanesthetized state while the monkeys sat in restraining chairs

a   PRE CHALLENGE

b   POST CHALLENGE TERMINAL SHOCK

c   TERMINAL ISOELECTRIC PATTERN

FIG. 1 *Progressive EEG changes associated with shock after SEB administration to monkeys The prechallenge normal pattern (a) persisted until after blood pressure had fallen to below 45 to 55 mm of Hg At that pressure, there was an alteration, to the pattern shown in b, which progressed to an isoelectric pattern (c) just prior to death*

to which they had become adapted After obtaining base line control tracings (Fig. 1a) for at least 30 min, seven monkeys were challenged iv with 1,000 μg of SEB per kg of body weight, and the eighth monkey was bled following anesthetization. Five of these challenged monkeys were given epinephrine (1:1,000, 1 ml, iv) after abnormal EEG patterns were noted

RESULTS

Regardless of the time to death (which ranged from 1 to 88 hr after challenge in those not treated), there were essentially no changes from control patterns in the EEG until 30 to 90 min before death. This phenomenon was observed in all monkeys given SEB, whether they were studied with surface electrodes during anesthesia or with dural electrodes in the nonanesthetized state. With the development of terminal clinical symptoms, which included cyanosis, severe depression, and shock, there was a marked decrease in wave frequency and an initial increase in amplitude (Fig. 1b). The frequency remained depressed while the amplitude decreased gradually, reaching an isoelectric pattern (Fig. 1c) just prior to death.

Epinephrine was administered to five monkeys after gross EEG changes were noted. Simultaneous arterial blood pressure recordings in three of these animals indicated that the pressure had dropped to levels of 45 to 55 mm of Hg before the EEG pattern was affected. After the injection of epinephrine, there was an immediate increase in blood pressure to normal or above normal levels. Other studies in our laboratory have shown that cardiac output is returned to values that are normal or above normal from approximately 40% below normal after the injection of epinephrine in the terminal stages of SEB toxemia. With such treatment, carotid artery flow is restored from approximately 50% of prechallenge values transiently to normal (*unpublished data*). Four of the monkeys showed definite improvement in their EEG; the fifth died immediately after epinephrine was injected.

Of the four that showed improvement, one had a silent EEG for 7 min before epinephrine injection. Although this EEG never returned to normal, cortical electrical activity definitely reappeared and produced the pattern shown in Fig. 2a. In contrast, there was no return of activity in the untreated animals once the isoelectric pattern was reached, and the animals soon died. The other three animals were treated with epinephrine as soon as patterns characterized by high-amplitude and low-frequency waves were noted. In these animals, the EEG returned to apparently normal patterns (Fig. 2b). This state could be maintained for only a few hours by a continuing epinephrine infusion, then preterminal EGG
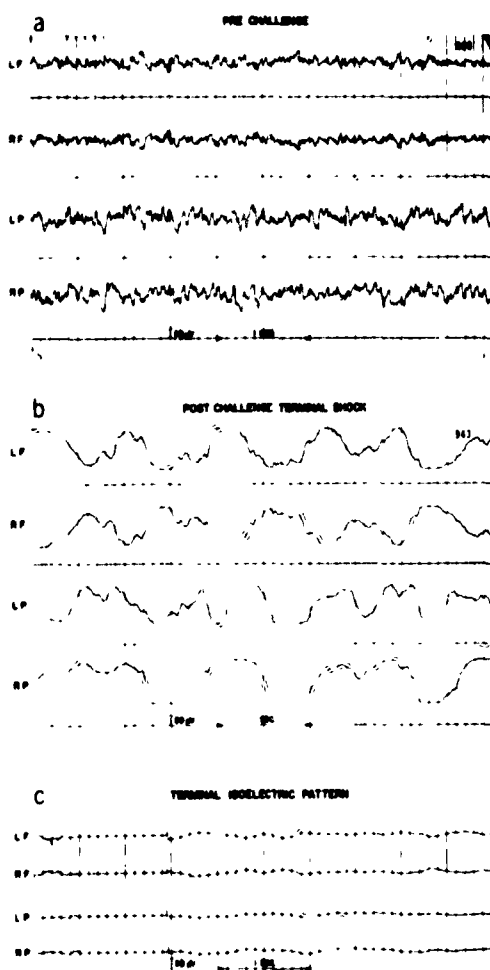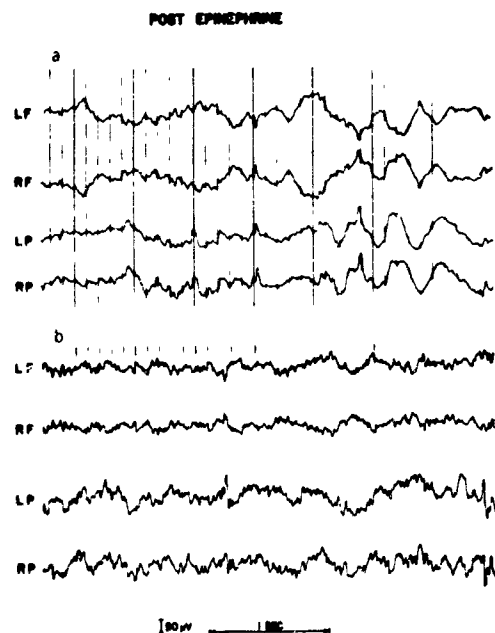
POST EPINEPHRINE

FIG. 2. *Transient improvement in EEG associated with epinephrine administration. The upper tracing (a) shows an improved pattern in an animal which had progressed earlier to an isoelectric pattern. (b) Return to an apparently normal pattern accompanied treatment when administered during the high-amplitude, low-frequency pattern.*

patterns, as previously described, reappeared, and the animals expired. Although epinephrine was not capable of preventing death from SEB toxicity after a challenge of 1,000 μg/kg, it increased blood pressure, cardiac output, and carotid artery flow in the terminal stage of SEB shock.

Because the observed changes in the EEG occurred synchronously with shock, control studies designed to mimic SEB shock by gradual removal of whole blood were performed. A 4- to 7-ml sample of whole blood was removed from each of two anesthetized monkeys every 0 5 hr. The cannula was then flushed with 1 ml of heparinized saline after each bleeding. No alterations in EEG were noted until the monkeys had lost large proportions of their circulating blood volume (75 ml in one, 41 ml in the other). After this point, only 2 ml was withdrawn each 0.5 hr. There was an immediate impact on the EEG after withdrawal of each of these additional samples, characterized by a pattern similar to that observed in the late stages of SEB toxemia; however, the pattern returned to normal until 87.5 ml had been removed from the first monkey and 52 0 ml from the second. After this, the EEG failed to return to normal, a per-sistent isoelectric pattern developed, and the monkeys expired 39 and 16 min later, respectively.

DISCUSSION

The EEG is being utilized with increasing frequency as an indirect method of monitoring cerebral blood flow during cardiac bypass procedures. Alterations in the EEG pattern similar to those observed during the terminal stages of SEB toxemia have also been reported following severe hypotension or cerebral hypoxia in humans and dogs (2, 3, 6, 7, 8, 13).

Cliffe et al. (3) stated that marked hypotension from any cause will produce changes in the EEG which are constant and reproducible. In general, the critical level at which gross changes occur in the EEG is 50 mm of Hg (1, 11), but this is dependent upon the nature and rate of pressure decrease. Vick (Federation Proc. 23:539, 1964) reported that no changes in the EEG occurred in dogs bled to a blood pressure level of 50 mm of Hg and maintained at that level for 10 hr.

Epinephrine, which has been shown to increase blood pressure, cardiac output, and carotid artery flow transiently to normal or above normal levels in the later stages of SEB toxemia, causes temporary improvement in the EEG. These observations imply that the iv challenge of monkeys with SEB does not produce detectable direct alterations in the cortical electrical activity.

Since alterations in the EEG occur only in the terminal stage of toxemia, regardless of the time after challenge, and, since an apparent increase in blood flow to the brain reverses these changes, it is postulated that cerebral anoxia, caused by inadequate blood flow, is the primary cause of the EEG pattern alterations produced by SEB. In this respect, SEB apparently is similar to the lipopolysaccharide, bacterial endotoxin, but it is distinctly different from the reported effects on cortical electrical activity of botulinum toxin (Type A), crude anthrax toxin, or crude rattlesnake and cobra venom.

The recording techniques employed in this study permitted evaluation of electrical activity of the cerebral cortex only. It remains possible that SEB might produce effects on deeper structures of the central nervous system, which could not be detected by surface electrodes.

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