Observations on Electrocorticographic Effects of Acetylcholine in Monkeys and Cats. (20176)

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(Introduced by W. H. Marshall.)

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Topical application of acetylcholine to the cerebral cortex of the cat and monkey has been reported to produce a local cortical depression, paroxysmal electrical discharges, and a spreading type of cortical depression(1-5,7, 8,12). We have repeated these experiments adding technics developed in this laboratory for the identification and control of spreading cortical depression of Leão(9-11). We have confirmed the local depressant and paroxysmal discharge effects of acetylcholine, but a spreading depression type of response was not obtained.

Method. Nine cats, anesthetized with nembutal 30 mg/kg or dial 30 mg/kg combined with nembutal 30 mg/kg, were used. One cerebral hemisphere was exposed and the electrocorticogram was recorded by means of 4 serially aligned wick electrodes usually placed on the suprasylvian gyrus. Each electrode recorded local D. C. potential (in mv range) in addition to brain wave activity(9). A single common reference electrode was placed on a saline moistened cotton pledget covered with mineral oil in contact with an area of exposed skull, this area was surrounded by a wall of dental plaster. In most cases arterial blood pressure was recorded from the femoral artery with a mercury manometer. Acetylcholine chloride (2% to 20%) was applied on filter paper pledgets approximately 4 mm square. The drug was also applied in droplet form from a No. 27 hypodermic needle to pledgets already in place. Care was taken to avoid mechanical stimulation of the cortex when these drugs were applied, and the cortex was maintained in a state as near physiological as possible for this type of experiment(10). The majority of trials were with 5 and 10% solutions. Similar experiments were done on 3 monkeys being used primarily for other experiments.

Results. Acetylcholine chloride produced several different electrocorticographic effects. These reactions were unpredictable and ranged from transitory local depression, through no discernible reaction, to a sustained convulsive type of activity. Repeated attempts to elicit propagated or remote effects by applying acetylcholine at finite distances from the recording electrodes met with failure. In contrast, application of the drug to pledgets monitored by an electrode failed to produce an electrocortical effect in only 3 of 48 trials (6%).

In accord with Forster and McCarter(5), it was noted that acetylcholine often induced a transitory diminution of activity followed by signs of hyperactivity. However, in contrast to the findings of Forster(3) and of Forster, Borkowski and McCarter(4), the temporary diminution in brain wave amplitude was local and static rather than migratory. In addition, the local depressant action was not associated with a significant or consistent D. C. voltage variation of magnitudes comparable with those observed in spreading depression of Leão. There was considerable scatter in the patterns of reaction. Isolated amplitude reduction with no subsequent evidence of increased activity occurred in 31% of trials. Approximately 19% of pledget applications resulted in local amplitude increase with no antecedent depression. In 44% of trials there was an immediate amplitude reduction followed within 5 to 10 minutes by increased electrical activity. Thus approximately 63% of trials showed signs of hyperactivity. Others have reported excitatory reactions in 58% of trials. The addition of Locke-Ringer solution to a pledget previously interposed between electrode and cortex failed to produce any of the electrical changes described above. Shunting, therefore, would not explain the temporary reduction in electrical activity.

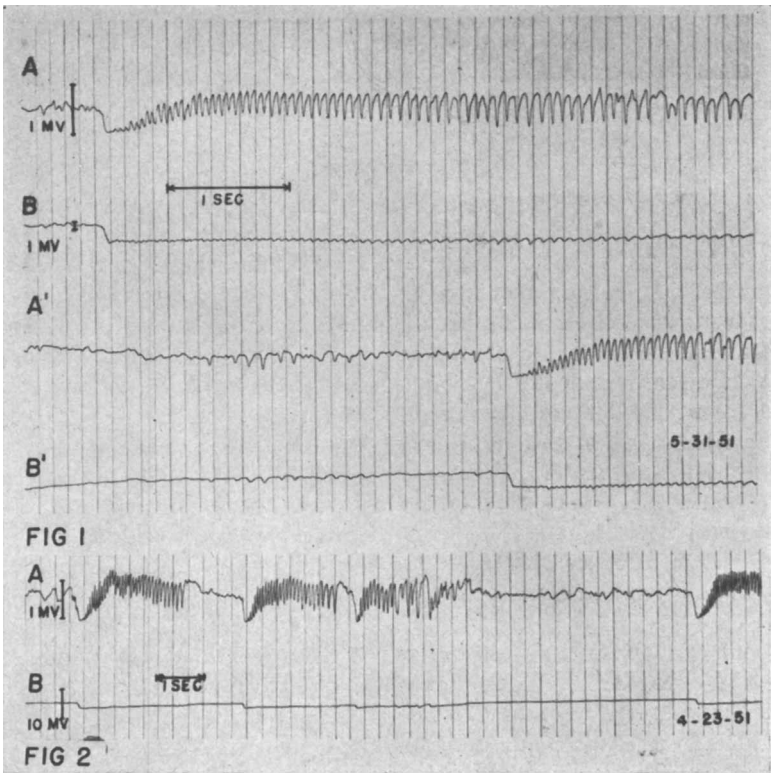

FIG. 1. Example of pulse-burst activity evoked by topical application of 10% acetylcholine to cortex of cat. A and B are records from the same electrode: Record A—through reactive coupled amplifier at sensitivity shown; record B—through direct coupled amplifier at approximately 0.1 sensitivity of record A. A′ and B′ are continuous with A and B reading time left to right. Record A-B has been cut and the contiguous section A′-B′ is mounted below it. This sample of record taken from dorsal striate 10 min. after application of 10% acetylcholine to area around electrode. Positive deflection is up in all records.

FIG. 2. A second example of this type of acetylcholine reaction from another cat. This record is from the mid-dorsal suprasylvian gyrus 16 min. after topical application of 10% acetylcholine.

In about 25% of 60 applications of acetylcholine the high amplitude activity was organized into brief bursts, each sequence of which was accompanied by a D. C. base line shift of 0.5 to 3.0 mv in the surface negative direction (Fig. 1). This burst sequence can be triggered by strychnine spikes produced by application of strychnine to the same region.

Local application of acetylcholine to the cerebral cortex usually produces a transient fall in systemic blood pressure. Hoff *et al.* (6) have reported similar results with methacholine. In agreement with Forster *et al.*(4) we also found that the transient fall in blood pressure was not the cause of the transient diminution of brain wave activity.

The electrocortical alterations produced by local acetylcholine application were less well defined in the monkey, but were otherwise basically similar. All effects are purely local, and various forms of acetylcholine discharges were seen in about 38% of trials.

Discussion. The somewhat variable results obtained in these experiments warrant only limited discussion. In summary, acetylcholine produces a gamut of purely local electrocortical alterations when applied to the exposed cerebral cortex of anesthetized cats and monkeys. No variety of spreading depression type of reaction was obtained. The concurrent incidence of burst spiking and a D. C. voltage shift is an intriguing phenomenon. However, the variability of the D. C. shift amplitudes makes causal interpretation difficult. The long

duration of the acetylcholine effects indicates the complexity of the reaction since the acetylcholine must be rapidly split by the esterase in the cortical tissue.

Summary. 1. Topical application of acetylcholine usually produced a local depression. The depression was not propagated, and was not due to changes of systemic blood pressure. 2. The local depression was often succeeded and sometimes masked by enhanced electrical reactivity. This action varied from a barely discernible increase to a sustained high voltage pattern lasting 30 minutes. The discharges were randomly distributed in time in some instances; in others, the activity was organized in distinct groups of paroxysmal spikes each group being accompanied by a distinct surface negative D. C. pulse.

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Relationship of Native Heparin to Clearing of an Alimentary Lipemia.* (20177)

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The dramatic *in vivo* clearing by commercial heparin and heparin-like substances of an alimentary lipemia has been noted by a number of workers(1-5). This clearing has been shown to be reversible in dogs and rats by injections of protamine(6), a substance which is known to combine with and inactivate heparin(7). In the intact dog it has also been shown that the clearing produced by small amounts of intravenous heparin is not initiated in the circulation of the brain, but is initiated in the circulation of the leg and lung; once circulation is complete and mixing of the blood has occurred clearing continues in samples of blood from all of these sources(8).

To test the effects of native heparin on an alimentary lipemia, anaphylactic shock and shock following the injection of certain histamine liberators have been studied. In other experiments the effects of intravenous injections of dextran have been observed. These studies will now be reported.

Material and methods.† Dogs were sensitized with 50% eggwhite (1 ml/kg). The initial injection was given intravenously and 5 and 7 days later this dose was repeated subcutaneously. Thirty days later the dogs were fed cream fat (2-4 g/kg body weight), and 3-4 hours later anaesthesia with nembutal was induced. A control lipemic sample of blood was then drawn. The small shock dose of antigen was injected intravenously. Four

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