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Comparison of the effects of bicuculline and strychnine on brain stem auditory evoked potentials in the cat

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1 Experiments were performed to determine the effects of intravenously applied bicuculline and strychnine on the click-evoked brain stem auditory evoked potentials (BAEP) of cats.

2 The BAEP was not affected by bicuculline (0.5 mg/kg, i.v.) administration. Strychnine (0.2 mg/kg, i.v.) administration caused a significant increase in the amplitude of peak 4, which is thought to be produced by potentials in the superior olive, lateral lemniscus and inferior colliculus.

3 These results suggest that strychnine blocks glycinergic inhibitory inputs to these auditory structures.

Introduction

The brain stem auditory evoked potential (BAEP) has been studied extensively in laboratory animals and man. The signal is generated by applying an acoustic stimulus to the ear and the resulting potentials at the vertex are measured. Field potentials produced by each relay nucleus along the auditory pathway are sufficiently separated in time that when the volume conducted potentials are averaged several distinct peaks are seen. The BAEP has been characterized by lesion and field recording experiments (Jewett, 1970; Buchwald & Huang, 1975; Achor & Starr, 1980; Borg, 1981) and there is general agreement that each of the first five peaks are associated with brainstem auditory relay nuclei.

There is considerable biochemical and pharmacological evidence that γ-aminobutyric acid (GABA) and glycine may act as neurotransmitters in auditory brainstem structures (cf. Martin, Dickson & Fex, 1982). Recently Zarbin, Wamsley & Kuhar (1981) reported strychnine binding in the superior olive, lateral lemniscus, inferior colliculus and the dorsal cochlear nucleus. In the experiments presented here the BAEP was used to determine whether bicuculline, a GABA antagonist (Curtis & Johnston, 1974), and strychnine, a glycine antagonist (Curtis & Johnston, 1974), alter the BAEP.

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Methods

Experiments were performed in a sound-proofed room. Seven adult (2.25 – 5.0 kg) male and female cats were anaesthetized with pentobarbitone (40 mg/kg). Anaesthesia was maintained with a 5 mg/ml solution of pentobarbitone given via a catheter in the brachial vein by periodic injection and constant infusion (1.5 ml/h). Body temperature was maintained between 36 and 38°C with a thermostatically controlled heating pad.

The external ear was removed, the tympanic bulla opened and its septum removed to expose the cochlea. Acoustic stimuli were presented in a closed sound system (Kiang, Watanabe, Thomas & Clark, 1965) through a 2.54 cm condenser microphone (Bruel and Kjaer). A wire electrode was placed adjacent to the round window (reference electrode on the tongue) to assess the threshold for click-evoked electrical activity.

For recording the BAEP a stainless screw was fixed at the lambda (junction of the lamboid and sagittal sutures) and reference wire electrode placed near the tympanic membrane in the lower caudal quadrant of the portion of the temporal bone forming the external meatus. Clicks (0.1 ms, 1.0 V) were presented at 10 per second. Signals were amplified by a Grass P511 amplifier. The bandpass was 10 Hz to 10 KHz and the gain was 50,000. Responses to 600 consecutive stimuli were averaged by a PDP 11/40 computer.

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Responses to click stimuli 0, 5, 10, 20 and 40 dB above the threshold for the compound action potential measured at the round window (referred to as 0 dB SL) were averaged. Three control responses to 600 consecutive stimuli each, at 10 min intervals, for each stimulus intensity were measured before bicuculline hydrochloride or strychnine sulphate was given intravenously. Doses of 0.1 or 0.2 mg/kg of strychnine were given to 4 animals. In 3 of these animals bicuculline, 0.2 or 0.5 mg/kg, was given intravenously, at least 65 min before the injection of strychnine. The drugs dissolved in saline were given as a single dose by slow infusion over a 4 min period. BAEP responses to each stimulus intensity were monitored for 60 min after bicuculline administration, and 30 to 300 min after strychnine administration.

In order to observe spontaneous variations in the BAEP over time, three control animals, which received no injections of test drugs, were also studied. As in the animals injected with convulsants, the average of the first 3 BAEP responses of 600 consecutive stimuli each, were used in calculating the percentage change with time. BAEP responses were then sampled at 15 min intervals for the first hour and 30 min intervals for the second. In one animal control values at 4 h and 6 h were also computed.

Results

In control animals the latencies of the individual peaks remained constant with time. For a 20 dB SL stimulus the latencies (ms) for the individual peaks were (mean ± s.d.) peak $1 = 1.51 \pm 0.08$, peak $2 = 2.67 \pm 0.10$, peak $4 = 4.49 \pm 0.19$, and $5 = 5.15 \pm 0.21$. Peak 3 was not visible with this recording arrangement at 20 dB SL. At 40 dB SL peak 3 appeared as a small inflection on the descending phase of peak 2. It was observed that the amplitudes of each peak varied over time but always within 20% of initial control values, even over an 8 h period.

Bicuculline (0.5 mg/kg) did not affect either the amplitude or latency of peaks 1, 2, 4 and 5 at any stimulus intensity or at any time interval. The shortest time interval measured was 5 min from the start of the bicuculline injection in each animal.

Strychnine, however, did alter the amplitude of peak 4 without significantly affecting peaks 1, 2 or 5. The effects of intravenous bicuculline and strychnine on the amplitude of peak 4, evoked by a 20 dB SL stimulus, are illustrated in Figure 1. For comparison, the variation in the amplitude of peak 4 in a control animal is also plotted. Within 10 min after the start of the strychnine injection the amplitude of peak 4 increased in all 4 animals. Following a dose of

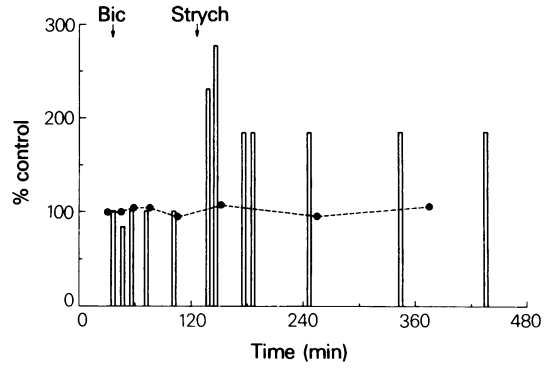

Figure 1 The effects of intravenously-applied bicuculline (Bic, 0.5 mg/kg) and strychnine (Strych, 0.2 mg/kg) on peak 4 of the brainstem auditory evoked potential (BAEP, 20 dB SL click). Columns and dashed line represent the percentage control for peak 4 for an animal injected with the convulsants and a control animal, respectively. The abscissa scale is the time from computation of the initial control BAEP average. The ordinate scale is percentage change relative to initial control values.

0.1 mg/kg, strychnine caused variable increases in the amplitude of peak 4 at 20 dB SL. In one animal the increase was to 128% (compared to intra-animal control) and in the other, to 211% of control values. In the two animals given 0.2 mg/kg, strychnine increased the amplitude to 277% and 231%, respectively. At stimulus intensities of 20 and 40 dB SL the increased amplitude of peak 4 was statistically significant (t test, $P < 0.005$) when compared to intra-animal controls and to controls at approximately the same post-surgical time. Occasionally the amplitude of peak 4 continued to increase for the next 10 min before it began to decline. At lower stimulus intensities the alterations in peak 4 were less evident. The latencies of all peaks remained unchanged after the strychnine injection.

Discussion

The results of these experiments indicate that the GABA-antagonist bicuculline had no effect on the BAEP of cats. This is consistent with the observation that neither diphenylhydantoin nor benzodiazepines affect BAEPs (Stockard, Rossiter, Jones & Sharbrough, 1977). Both are believed to modulate GABA-induced responses (Straughan, 1979; Olsen, 1981; Simmonds, 1981). Penicillin on the other hand is a GABA antagonist and also has no effect on the BAEP (Stockard *et al.*, 1977). Pentobarbitone potentiates GABA responses (Straughan, 1979). If GABAergic synapses are involved in the generation

of the BAEP, pentobarbitone would be expected to depress the BAEP. However, pentobarbitone has no effect on the BAEP (Stockard *et al.*, 1977; Bobbin, May & Lemoine, 1979). Even though pentobarbitone may not affect the BAEP, it may interfere with the action of bicuculline. As pentobarbitone potentiates the action of GABA it reduces the potency of bicuculline (Evans, 1979). However, in animals deeply anaesthetized with pentobarbitone, a dose of 0.5 mg/kg of bicuculline, as used in this study, is effective in antagonizing GABA-mediated inhibitions (Curtis, Duggan, Felix & Johnston, 1971). Thus the results from this study on the actions of bicuculline are consistent with the results from other studies, all of which suggest that GABAergic mechanisms are not involved in the generation of the BAEP.

The results also show that strychnine significantly increases the amplitude of peak 4. Peak 4 is believed to be generated primarily in the superior olive, lateral lemniscus and inferior colliculus (Jewett, 1970; Buchwald & Huang, 1975; Achor & Starr, 1980; Borg, 1981). Strychnine has been shown to be an antagonist of glycine-like neurotransmitters (Curtis & Johnston, 1974). Strychnine-sensitive (glycinergic) inhibitions tend to be restricted to the brainstem and spinal cord while bicuculline-sensitive (GABAergic) inhibitions prevail in the cerebrum and cerebellum (Curtis & Johnston, 1974). Strychnine-sensitive, bicuculline-insensitive, inhibitions have been described in the superior olive (Moore, Caspary & Havey, 1981). Also, high levels of strychnine binding are observed in the superior olive, lateral lemniscus and inferior colliculus (Zarbin *et al.*, 1981). This suggests that the increased amplitude of peak 4 resulting from intravenous injections of strychnine is due to antagonism of tonic or click-evoked glycinergic inputs to these structures. The origin and function of the glycinergic inhibition are unknown; their identification will require a detailed physiological and pharmacological analysis of single unit activity.

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