BLOCKADE BY ESERINE OF THE CEREBRAL CORTICAL EFFECTS OF ALLOFERIN: A FURTHER EVIDENCE OF CHOLINERGIC INHIBITORY MECHANISM

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Abstract—In rats anaesthetised with pentobarbitone, somatosensory evoked potentials, produced by stimulation of contralateral or ipsilateral forepaws were used to measure the activity in different neuronal pathways. Computer derived averages of 32 consecutive responses yielded a stable and consistent measurement of the evoked potentials. The drugs were applied to the cerebral cortex by using cortical cup technique and changes were measured in evoked potentials. Cortical application of alloferin increased the amplitude of the early negative wave of the cortical evoked potentials. This effect of alloferin was blocked by prior treatment of the cortex with eserine. It appears that the excitant effect of alloferin on the cortex was the result of disinhibition of cortical cholinergic inhibitory mechanism.

In recent years, cortical inhibition has received increasing attention from Neurophysiologists and Pharmacologists. There is considerable evidence of an 'Inhibition blockade' mechanism for the excitant effect of strychnine, curare and atropine (1, 2). The neuro-humoral mechanism involved in cortical inhibition is still however debatable. Application of alloferin to the exposed cerebral cortex leads to abnormality in the somatosensory evoked potentials after a few minutes (1). This consists of a surface negative wave (peak latency 21–23 msec) which attains an amplitude five to ten times greater than that of the normal evoked potentials, probably reflecting an excessive depolarization of the apical dendrites of pyramidal neurones. This effect of alloferin is similar to that of strychnine or curare (1). The effect of strychnine is blocked by eserine (3), suggesting that the excitant effect of strychnine occurs as a result of disinhibition of the cholinergic inhibitory mechanism in the cortex as described by Phillis and York (4, 5). The present study was undertaken to study the blocking of strychnine like action of diallyl nortoxiferine dihydrochloride (alloferin) by eserine to further strengthen the hypothesis of cortical cholinergic inhibitory mechanism.

MATERIALS AND METHODS

Male rats of CFE strain (Sprague-Dawley from Carworth, Europe) weighing 250–300 g were anaesthetised with pentobarbitone (50 mg/kg initially, then 12 mg/kg every 30 min). By the technique of Bhargava and Meldrum (1) animals were prepared for the recording of cortical somatosensory evoked responses. Computer derived averages of 32 consecutive somatosensory evoked potentials from both cortices, in response to the

stimulation of contralateral and ipsilateral forepaws were recorded. Drugs were applied to the pial surface by injection through polythene tubing connected to a perspex ring and removed by multiple washing with artificial cerebrospinal fluid (CSF) (6). Drugs were freshly dissolved in CSF at 37°C for each experiment. The drugs were applied to one cortex while the other cortex was bathed with normal CSF and served as control throughout the experiment. In each experiment, the effect of a 15 min application of alloferin was initially tested on left and right cortex. After 1 hr recovery from the effect of alloferin, 10^{-3} M eserine sulphate was applied to one cortex for 60 min and changes in the evoked potentials by eserine were studied. Alloferin was reapplied to the eserine treated cortex to determine whether or not eserine modified the effect of alloferin.

A continuous polygraphic record of cortical and rectal temperatures was taken by thermocouples.

RESULTS

Normal form of somatosensory evoked potential under pentobarbitone

An afferent volley from a peripheral nerve evokes a diphasic potential on the surface of the somatosensory cortex. The potential changes in the primary receiving area in the first 50 msec after contralateral stimulus show an initial positive wave (P_1) with a peak at 7 ± 1 msec ($N=65$) and subsequent one (P_2), at 11.5 ± 1.8 msec ($N=65$), followed by a longer lasting negative wave (N_2), peak at 40 ± 6.2 msec ($N=65$). Some records show a sharp early negative wave (N_1) with peak latency at 16.3 ± 2.9 msec ($N=27$), as reported earlier (1).

Effect of alloferin

Cortical application of CSF containing various concentrations of alloferin for 15 min constantly led to an appearance of an abnormally large surface negative wave (peak latency 21–23 msec). The threshold concentration was 3×10^{-4} M, which produced an effect after 15–20 min. 2×10^{-3} M alloferin produced an increase in the early negative wave of the somatosensory evoked potential in 5.5 ± 0.7 min, which became maximal after 13.5 ± 2.1 min and lasted for 42.5 ± 3.5 min after washing out the drug. The increase in the amplitude of the early negative wave after 3×10^{-4} M was 0.5 ± 0.2 mV, 10^{-3} M 2.0 ± 0.4 mV and after 2×10^{-3} M alloferin was 3.6 ± 1.3 mV. A direct linear relationship between log concentrations and the amplitude of the early negative wave was seen.

The first positive wave constantly showed no change in latency amplitude or duration after alloferin. The second positive wave however, at times showed a slight increase in the amplitude which was not statistically significant. On the control cortex the cortical responses to contralateral stimulation showed no changes. However, a biphasic positive negative wave on ipsilateral stimulation appeared when the drug treated hemisphere showed an abnormal negative wave. This wave reversed at a depth of 1–1.5 mm below the surface when recorded by subcortical electrode.

Blockade by eserine of the cortical effect of alloferin

In some experiments, in order to determine whether or not the strychnine-like effect

FIG. 1. (a) Effect of cortical application of alloferin 2×10^{-3} M, (b) the same cortex 1 hr later. The effect of eserine 10^{-3} M, followed by alloferin 2×10^{-3} M. The 400 μ V calibration applies to the traces in at times alloferin 15 min to wash 10 min. Each trace is the average of thirty-two consecutive responses recorded during 130 min after stimulation of the contralateral forepaw. Positivity of the electrode at the somatosensory cortex is shown upwards. The 100 μ V calibration applies to all other traces.

TABLE 1. Effect of cortical application of alloferin (15 min) on amplitude of the early negative wave (N1) (peak latency 22 msec) of the cortical evoked potentials before and after prior treatment of the cortex with eserine sulphate (10^{-3} M) for 60 min.

Conc. of drug	Amplitude N1 (mv) control	Amplitude N1 (mv) drug	Amplitude N1 (mv) eserine	Amplitude N1 (mv) drug	No. exp.	% Inhibition
3×10^{-4} M	0	0.48 $\pm$ 0.21	0	0	5	100%
10^{-3} M	0	2.0 $\pm$ 0.4	0	0.5 $\pm$ 0.4	5	75%
2×10^{-3} M	0	3.6 $\pm$ 1.3	0	1.2 $\pm$ 0.3	5	66%

of alloferin was the result of action on the cholinergic inhibitory system; the effect of 15 min application of different concentrations of alloferin was initially tested on the left and the right somatosensory cortex. After one hour recovery from the effect of alloferin, 10^{-3} M eserine sulphate was applied to one cortex for 60 min. Eserine itself did not modify the cortical evoked potentials, however, it did block the effect of alloferin on the early negative wave of the cortical evoked potentials. The effect of 3×10^{-4} M alloferin was blocked by 100%, that of 10^{-3} M by 75% and of 2×10^{-3} M by 66%. (Table 1).

DISCUSSION

Electrical stimulation of the forepaw of the rat under pentobarbitone produces biphasic positive negative potentials at the primary receiving area. These positive negative waves are mostly derived from differences in potentials between portions of cortical

neurons situated at different levels. The surface positive waves are considered to represent activity in the depth of the cortex while early negative wave represents conduction of the response towards the cortical surface, presumably as a result of depolarization of apical dendrites of pyramidal neurons (7). The present study shows that cortical application of alloferin results in appearance and augmentation of a surface negative wave (peak latency 22 msec) of the primary cortical evoked potentials. The effect is closely similar to that of curare, strychnine and cobra venom (1, 8). A common mechanism of action would, therefore, appear to be responsible for the characteristic action of alloferin, curare and cobra venom. This could be disinhibition of the cortical cholinergic inhibitory mechanism. The strychnine like effect of alloferin was blocked by prior application of eserine to the cortex. One possible interpretation of this antagonistic effect of eserine is that alloferin blocks a cholinergic inhibitory mechanism in the cortex, which normally prevents massive depolarization of the apical dendrites of pyramidal neurons. Phillis and York (4, 5), have shown an inhibitory mechanism acting on the cells in layers II, III and IV in the cortex. The cells are inhibited by cholinomimetic drugs and blocked by strychnine, curare and atropine. Thus this inhibitory mechanism and an enhancement of negative wave of cortical evoked potentials seen in present experiments have the same drug sensitivity. It appears that alloferin and related drugs produce their excitant effect by blocking cholinergic inhibitory mechanism.

The phenomenon of cortical inhibition is an unresolved problem and the exact mechanism and site of action of cortical excitant drugs remains to be elucidated until the identity of the cortical inhibitory transmitter is known.

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