

NEUROPHARMACOLOGICAL STUDIES ON A NEW SERIES OF ERGOT ALKALOIDS THE EFFECTS ON ELECTROCORTICOGRAM OF RABBITS

TOHORU YUI AND YUJI TAKEO

Research Laboratories, Takeda Pharmaceutical Industries, Ltd., Osaka*

Received for publication December 6, 1957

Lysergic acid diethylamide (LSD), a compound derived from ergot alkaloids, produces in human subjects a characteristic mental disturbance with visual hallucinations and other psychotic symptoms in a very small dose. Studies of the effects of LSD on spontaneous cortical activity in animals have been reported by a number of investigators. Bradley and Elkes (1) reported that LSD produced rhythms not unlike those seen in the normal alert animal, i.e., low amplitude, diffuse fast (15 to 30 c/sec) activity in all regions of the brain of the conscious cats. Rinaldi and Himwich (2) studied the effects of LSD on the spontaneous cerebral activity in curarized rabbits and found that it caused a continuous "alert pattern" in small doses (10 to 15 $\mu\text{g}/\text{kg}$).

Recently, Yui and Takeo (3) reported on central actions of a new series of ergot alkaloids isolated by Abe *et al.*. The excitor group among these alkaloids produced in various species of animals characteristic symptoms with hyperpnoea, mydriasis, increase in the spontaneous activity and anxiety which were similar to those induced by LSD, and antagonized markedly reserpine-sedation. Moreover, some of these alkaloids antagonized some action of serotonin *in vitro* as well as *in vivo*. On the other hand, in contrast to the above-mentioned alkaloids, some of their dihydro-derivatives (the inhibitor group) produced in animals a syndrome probably attributed to depression of central nervous system.

From these results, it may be expected that these alkaloids produce certain electrophysiological changes of cerebral activity.

The experiments were performed on agroclavine and elymoclavine as representatives of the excitor group and dihydroagroclavine and dihydroelymoclavine of the inhibitor group.

This report is concerned with; 1) the effects of these four alkaloids on the spontaneous cortical activity of curarized or non-curarized rabbit in comparison with LSD, methylamphetamine and physostigmine, 2) how these EEG-effects are modified by some central depressants.

METHODS

Under thiopental anesthesia, three electrodes were screwed through the skull with the tips on the dura mater of the right frontal, parietal and occipital areas of the cortex of adult albino

* Address: Higashi-yodogawa-ku, Osaka, Japan

rabbits. A steel needle insulated with silicon except for the tip was used for the electrode. After 3 to 7 days, the cortical activity was recorded from bipolar leads, in most cases in the animals immobilized by intravenous injection of small doses of *d*-tubocurarine (less than 0.5 mg/kg/hr) and maintained by artificial respiration, and in some cases in the animals non-curarized and placed in the box in which they had been trained to rest in a normal crouching position. Recordings were made with a 2-channel, ink-writing electroencephalographic instrument.

External stimulation was effected by means of acoustic (bell) or tactile stimuli (touching or pinching the nose).

Agroclavine, elymoclavine, dihydroagroclavine and dihydroelymoclavine were used as solutions in 0.2% tartaric acid solution. Other drugs employed were; lysergic acid diethylamide (LSD), methylamphetamine, physostigmine sulphate, reserpine, chlorpromazine HCl and meprobamate. Most of the drugs were administered by intravenous route, except for meprobamate which was administered intraperitoneally as an arabia-gum suspension.

RESULTS

I. The normal electrocorticogram of curarized and non-curarized, unanesthetized rabbit

The normal pattern of the electrocorticogram of curarized, unanesthetized rabbit was characterized by high voltage (100 to 300 microV), slow (1 to 3 c/sec) waves and spindles of 10 to 15 c/sec waves in all records, when the animal was kept undisturbed (Fig. 1, A).

With external stimulation, very marked changes of activity were observed as appearance of "alert pattern." This pattern was characterized by disappearance of the slow waves and spindles, which were replaced by fast (20 to 30 c/sec), low-voltage (30 to 50 microV) waves, especially evident in the frontal-parietal lead. In the parietal-occipital lead, the pattern was obtained, which consisted of 5 to 6 c/sec waves of 40 to 60 microV with superimposed fast rhythms. This change normally lasted only for a few seconds after the end of the stimulation and was restored to original pattern (Fig. 1, B, C).

The normal electrocorticogram of non-curarized, unanesthetized rabbit consisted of the main rhythms of 4 to 8 c/sec at 40 to 60 microV which were intermixed with slow waves and spindles frequently (Fig. 1, A). With external stimulation, the same changes as in the curarized rabbit were observed.

II. The effects of agroclavine, elymoclavine, LSD, methylamphetamine and physostigmine on the electrocorticogram of curarized rabbit

Agroclavine: With small doses (0.05 mg/kg), the slow waves and spindles diminished but never disappeared completely. Larger doses (0.1 to 0.2 mg/kg) caused a very marked change of EEG, characterized by disappearance of the slow waves and spindles and persistent appearance of fast (20 to 30 c/sec) low-voltage (30 to 50 microV) waves in the frontal-parietal lead. In the parietal-occipital lead, there were waves of 5 to 6 c/sec at 40 to 60 microV with a superimposed fast rhythm. These changes were not distinguished from those obtained by

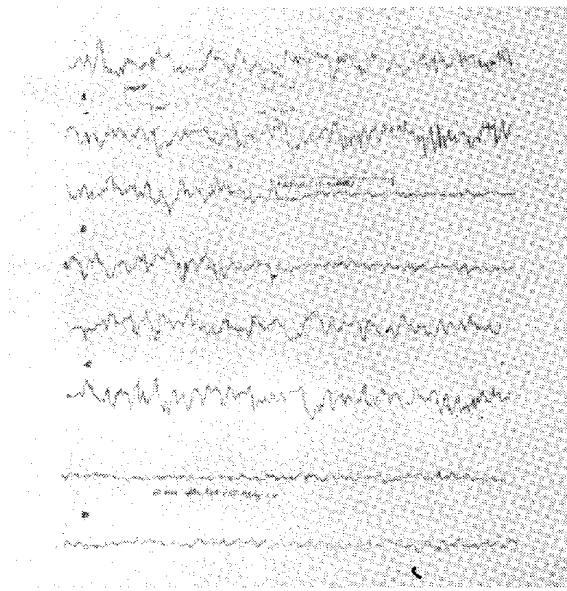


FIG. 1. Control pattern and the effect of external stimuli and agroclavine on the electrocorticogram of curarized, unanesthetized rabbit. Rabbit, 2.2 kg.

- A : Control pattern of the electrocorticogram
 B : Alert pattern induced by acoustic stimuli (bell at arrow)
 C : 10 sec after the end of acoustic stimulation
 Return from "alert pattern" to normal pattern
 D : The effect of agroclavine (150 μ g/kg i.v., 10 min after)
 Leads : Upper : Right frontal-Right parietal bipolar lead
 Lower : Right parietal-Right occipital bipolar lead

external stimulation, but lasted for 1 to 2 hours (Fig. 1, D).

Elymoclavine : Elymoclavine was found to produce similar effects to those of agroclavine in smaller doses (0.05 to 0.1 mg/kg).

LSD, methylamphetamine and physostigmine : LSD (0.01 to 0.03 mg/kg), methylamphetamine (20 to 30 mg/kg) and physostigmine (0.1 to 0.3 mg/kg) induced marked changes of EEG respectively, very similar to those induced by agroclavine and elymoclavine.

III. The effects of central depressants (chlorpromazine, reserpine and meprobamate) on agroclavine, elymoclavine, LSD, methamphetamine and physostigmine in electrocorticogram

By pretreatment with moderate doses of a central depressant, agroclavine, elymoclavine, LSD, methylamphetamine and physostigmine produced the EEG-effects respectively more or less different from those observed in unpretreated animal.

Chlorpromazine : Ten minutes after administration of moderate doses (0.5 to 1 mg/kg) of chlorpromazine, that changed the EEG in slight or negligible extent by itself, each of agroclavine, elymoclavine, LSD and methylamphetamine induced the decrease of the amplitude and the number of slow waves and spindles, but did not produce "alert pattern" which was seen in unpretreated animal (Fig. 2). On the other hand, the EEG effects of physostigmine

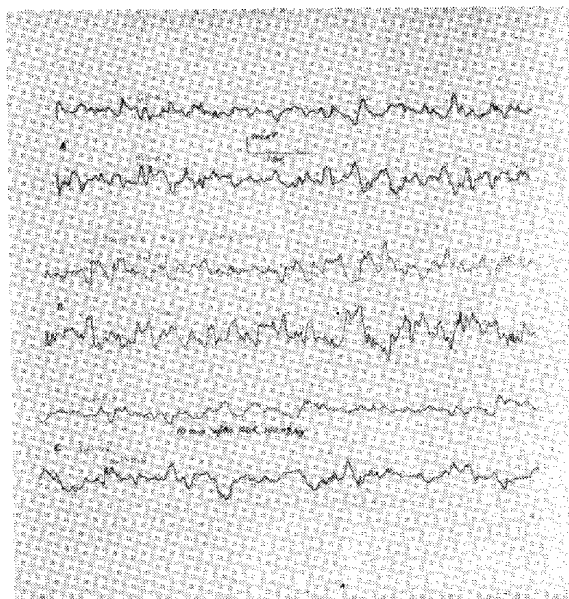


FIG. 2. Effect of chlorpromazine HCl on action of agroclavine. Curarized, unanesthetized rabbit, 2.3 kg.
 A : Control pattern of the electrocorticogram
 B : 5 min after chlorpromazine HCl, 1 mg/kg i.v.
 C : 10 min after agroclavine, 200 μ g/kg i.v. pretreating by chlorpromazine 5 min before
 Pretreatment with chlorpromazine fails to produce the typical pattern induced by agroclavine (cf. Fig. 1, D)
 Leads as indicated in Fig. 1

(0.1 to 0.3 mg/kg) were not affected even by pretreatment with larger doses (3 mg/kg) of chlorpromazine.

Reserpine: By pretreatment with reserpine (2 mg/kg) 5 hours before, some modification of the effects of agroclavine, elymoclavine and LSD was observed (Fig. 3), but those of methylamphetamine and physostigmine were hardly affected.

Meprobamate: By pretreatment with meprobamate (50 to 70 mg/kg) 30 minutes before, which induced the increase of the amplitude and the number of slow waves and spindles in some extent by itself, slight modification of the effects of agroclavine, elymoclavine and LSD was observed, i.e., the fast low-voltage waves intermixed with slow waves of intermediate amplitude (80 to 120 microV) were obtained. The effects of physostigmine were hardly affected by meprobamate.

IV. The effects of dihydroagroclavine and dihydroelymoclavine on the electrocorticogram of non-curarized, unanesthetized rabbit

Dihydroagroclavine: In doses less than 0.5 mg/kg, no noticeable changes of electrocorticogram were observed. Within 5 to 10 minutes following the injection of 0.5 mg/kg, an increase in the number and amplitude of slow waves (1 to 3 c/sec) and spindles (10 to 15 c/sec) was

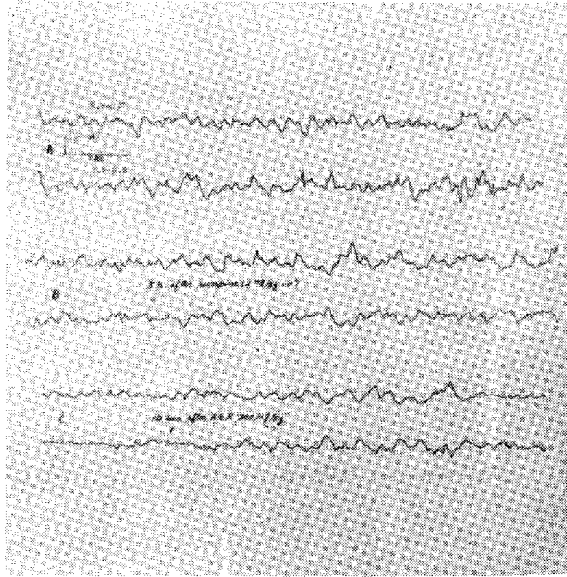


FIG. 3. Effect of reserpine on action of agroclavine.

Curarized, unanesthetized rabbit, 2.2 kg.

A: Control pattern of the electrocorticogram

B: 5 hrs after reserpine (2 mg/kg i.v.)

C: 10 min after agroclavine (200 µg/kg i.v.)

Pretreatment with reserpine fails to produce the typical pattern induced by agroclavine (cf. Fig. 1, D)

Leads as indicated in Fig. 1

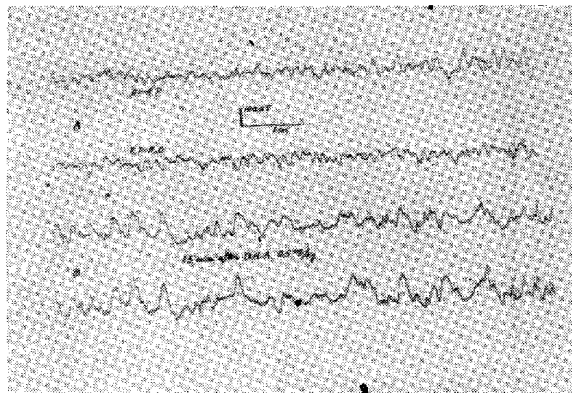


FIG. 4. Effect of dihydroagroclavine on the electrocorticogram of non-curarized, unanesthetized rabbit. Rabbit, 2.5 kg.

A: Control electrocorticogram pattern of non-curarized, unanesthetized rabbit

B: The effect of dihydroagroclavine (15 min after 500 µg/kg i.v.)

Leads as indicated in Fig. 1

noted (Fig. 4, B). These changes lasted for about 1 hour. During these changes, the normal response to external stimulation was obtained.

Dihydroelymoclavine: Within 5 to 10 minutes following the injection of 1 mg/kg, an increase of slow waves and spindles, interrupted by fast low-voltage waves occasionally, was noted.

DISCUSSION

These experiments have presented data on the effects of agroclavine and elymoclavine on the electrocorticogram of curarized rabbit and the effects of these alkaloids were very similar to those of LSD, methylamphetamine and physostigmine. All these drugs produced typical changes of spontaneous cortical activity which were undistinguished from those obtained by sensory stimulation. On the effects of LSD, methylamphetamine and physostigmine on EEG, our results were identical with those of many other reports [LSD (1, 2), methylamphetamine (4), physostigmine (5)]. The EEG-effects of agroclavine, elymoclavine and LSD were counteracted by pretreatment of chlorpromazine, reserpine and meprobamate respectively. However, the effects of methylamphetamine were hardly affected by reserpine though they were counteracted by chlorpromazine. Also, the effects of physostigmine were not affected by the doses used here of chlorpromazine, reserpine and meprobamate respectively. These results suggested that site of action of agroclavine or elymoclavine might be different from those of methylamphetamine and physostigmine but similar to that of LSD in some parts. It must be noted that there is a difference between amphetamine and agroclavine or elymoclavine in the interaction with reserpine on electrocorticogram, though both of them show a remarkable analeptic action on reserpine-sedated animal (3). These results will be elucidated by further electrophysiological investigation on the subcortical areas of the brain and evoked potentials.

According to Rinaldi and Himwich (6), King Killam and Killam (7) and Sailer and Stampf (8), reserpine had no effect on "arousal reaction" evoked by stimulation of the reticular activating system. Supporting their results, the counteraction by reserpine on the EEG-effects of agroclavine and elymoclavine suggests that their effects may be not due to direct action on the reticular activating system.

Agroclavine and elymoclavine resemble LSD not only in general behavior but in the effects on electrocorticogram. This finding indicates that both of them may cause some central actions in the same manner as LSD.

In contrast to agroclavine and elymoclavine, dihydroagroclavine and dihydroelymoclavine produced the resting pattern predominately which was characterized by slow waves and spindles. It may be interesting that dihydrogenation converts excitation to depression in central actions of ergot alkaloids.

SUMMARY

The effects of agroclavine, elymoclavine, dihydroagroclavine and dihydroelymoclavine on electrocorticogram of curarized or non-curarized rabbits were studied.

1. Agroclavine and elymoclavine produced continuous "alert pattern" undistinguishable from those obtained by external stimulation or administration of LSD, methylamphetamine and physostigmine in curarized rabbits.

2. The effects of agroclavine, elymoclavine and LSD were counteracted by pretreatment of chlorpromazine, reserpine and meprobamate. Those of methylamphetamine were counteracted by chlorpromazine but unaffected by reserpine. On the other hand, those of physostigmine were not affected by any of these central depressants.

3. Dihydroagroclavine and dihydroelymoclavine in non-curarized rabbits produced the slow waves and spindles predominatingly which were interrupted by fast low-voltage waves in some extent.

Acknowledgement. The authors wish to express their gratitude to Dr. *Kuwada*, Chief Director of Research Laboratories, Takeda Pharmaceutical Ind. for his active advice.

REFERENCES

- 1) BRADLEY, P.B. AND ELKES, J.: *J. Physiol.* **120**, 13P (1957)
- 2) RINALDI, F. AND HIMWICH, H.E.: *Science* **122**, 198 (1955)
- 3) YUI, T. AND TAKEO, Y.: *Jap. J. Pharmacol.* **7**, 161 (1958)
- 4) BRADLEY, P.B. AND HANCE, A.J.: *J. Physiol.* **129**, 50P (1955)
- 5) LONGO, V.G. AND BOVET, D.: *J. Pharmacol.* **120**, 160 (1957)
- 6) RINALDI, F. AND HIMWICH, H.E.: *Ann. N. Y. Acad. Sci.* **61**, 27 (1955)
- 7) KING KILLAM, E. AND KILLAM, K.F.: *J. Pharmacol.* **116**, 25 (1956)
- 8) SAILER, S. AND STAMPF, CH.: *Arch. exp. Path. Pharmac.* **230**, 378 (1957)