PHARMACOLOGICAL STUDIES ON THE FUNCTION OF THE EXTRAPYRAMIDAL SYSTEM

REPORT NO. 2

MECHANISM OF THE APPEARANCE OF TREMOR DUE TO EXTRAPYRAMIDAL POISONS

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INTRODUCTION

In the previous report(1) on some pharmacological properties of harmine and bulbo-capnine, these compounds were designated as extrapyramidal poison because of their specific actions. It was ascertained by further investigations with mice that the cerebral cortex, striatum, hypothalamus, and the ventral portion of midbrain are intimately connected with the appearance of the toxic effects of these compounds. Since the point of attack of harmine and bulbo-capnine are both localized and antagonistic in their actions, a separate and distinct reactive system for each may be conceived. While the harmine-reactive system has mainly to do with acceleration of the motor function, the bulbo-capnine-reactive system is concerned with its reverse effect. From the fact that tremor can be elicited as one expression of increased motor function produced by harmine, the present investigation is concerned with detailed studies on the nature and mechanism of such tremors.

Harmine is an alkaloid extracted from harmel seeds (*Peganum harmala*) whose general properties were studied by Flury(2) and Seto(3). With regard to its action on the extrapyramidal system, Hara(4, 5), Uehara(6, 7), Ino(8), and Tsukada(9) have published their observations. The effect of harmine on the appearance of tremor has been noted by others but little attention has been paid to the aspect of the point of attack. Ino(8) referred to the relationship between the tremor produced by harmine and excision of a cerebral hemisphere. There are also reports on the tremor effect of nicotine, eserine, and a few other chemicals but their tremor-producing effect is far less pronounced than that of harmine.

Tremor, because of its peculiar nature, can readily be distinguished from other motor symptoms and is a valuable sign indicating the appearance of the extrapyramidal action. Moreover, the analysis and elucidation of tremor will yield, it is believed, some important clues as to the functions of the extrapyramidal system.

FIG. 1 A. Tremor tracing apparatus used for mouse (by Method No. 1)

Straw lever, about 30cm, celluloid mouse chamber, 30 g.

MATERIALS AND METHODS

In the present series of experiments, harmine hydrochloride (Takeda) and bulbo-capnine hydrochloride (Merck) were used, together with other extrapyramidal poisons and poisons affecting the central nervous system. Mouse was selected as the experimental animal after various tests.

For the tracing of the movement, two mechanical and one electrical method were devised in order to record and observe tremor in the mouse, one or the other being

FIG. 1 B. Tracing of harmine tremor in mouse (by Method No. 1)

FIG. 2 A. Tremor tracing apparatus used for mouse (by Method No. 2)

Celluloid mouse chamber is fixed so that it cannot move vertically but only horizontally; movement of the chamber is magnified and traced by lever

employed as best suited to the occasion. The first tracing method (Fig. 1, A) utilizes the resonance of a straw lever which enables recording of only the vibration due to tremor in an amplified form on a smoked paper. The tracings taken by this method (Fig. 1, B) show gross motion, such as walking, as gentle waves of large amplitudes and tremors as fine waves of small amplitudes. The second tracing method (Fig. 2, A) makes use of a chamber which does not record gross movements, such as the transference of

a body by walking, but traces only fine movements, such as tremor. This is suitable for recording by means of a lever with a high magnifying rate to enlarge the amplitude and to trace only the change in tremor (Fig. 2, B).

FIG. 2 B. Tracing of harmine tremor of mouse (by Method No. 2)

The third method (Fig. 3, A) records only fine movements, such as tremor in the mouse, through oscillograms by electrical amplification. This method is suited for detailed recording of cycles of motion and wave forms (Fig. 3, B).

FIG. 3 A. Tremor tracing apparatus used for mouse (by Method No. 3)

Mouse chamber weighs 2 to 7 g. Tremor is converted into electric vibrations; amplitude being enlarged, and tracings taken by oscillograph

FIG. 3 B. Tracing of harmine tremor of mouse (by Method No. 3)

Destruction of the brain was carried out by coagulation by means of a high frequency current and by incision or excision with a specially-designed, fine scalpel. Destruction by high frequency current was carried out by passing current through needle electrodes thrust into the brain via a thermocouple ammeter from an oscillator of about 2 Mc/sec. The needle electrodes were fixed to simple holders which can be adjusted to reach any part of the brain. The parts destroyed were determined from histological specimens. Projection diagram of each of the subcortical nuclei was made from the histological specimens to determine the location and depth of the electrode penetration and the accurate position of the section destroyed.

RESULTS OF EXPERIMENTS

1) Comparison of Tremor-producing Action of Central Nerve Poisons and That of Harmine

The compounds which are known to produce tremor are harmine, harmaline, nicotine, eserine, and brucine. Nicotine and eserine produce appreciable tremor by subcutaneous injections of 0.02mg./10g., and 0.1 mg./10g., respectively. These are almost fatal doses and often produce tetanic convulsions. Other cerebral stimulants, such as picrotoxin, cardiazole, and strychnine, do not produce tremor even in tetanic or fatal doses. Caffeine, in a very large dose, produces a slight motion resembling tremor but no clear recording or observation could be made of such a motion. Even when marked excitation and increased movement are produced by methamphetamine or other stimulant amines, tremor is entirely absent.

On the other hand, as was observed by Uehara(6, 7) and Ino(8), harmine and harmaline produce severe tremor when injected subcutaneously (0.1—0.2 mg./10 g.) without the appearance of other serious symptoms. The minimum dose of harmine to produce the tremor is 0.01—0.02 mg./10g., subcutaneous injection of 0.1 mg./10g. resulting in the appearance of a marked tremor within a few minutes (Fig. 1, B). At the same time, a considerable reflex excitation develops and the mouse's tail becomes rigid to the touch. The reflex excitation due to harmine is slight compared to that produced by nicotine. The tremor produced by the administration of 0.1mg./10 g. of harmine lasts from 15 to 30 minutes. Administration of 0.2 mg./10 g. of harmine by subcutaneous injection produces such a severe tremor that walking is impaired. The amplitude of tremor in such a case is 2.0 cm. as recorded by the tracing method No. 1 and 4.0 cm. by the method No. 2 (Fig. 2, B). Compared to the lethal dose of 3 mg./10 g. of harmine, the tremor-producing action of this compound is evidently stronger than that of any other drug. This tremor-producing action of harmine can be observed in a slight degree by the intravenous administration of 0.01—0.02 mg./10 g., and in a marked degree by 0.05 mg./10 g., showing that there is only a very small quantitative difference in the route of administration. The tremor appears immediately after intravenous injection and lasts for 15 to 30

minutes which shows that only the lapse of time until appearance of tremor is different from that by subcutaneous injection, presumably due to the difference in the speed of absorption

Tracings by the third method show the movement of a normal mouse as irregular waves of a small amplitude of over 30 cycles per second. The curves of tremor appearing after the administration of harmine show a considerable increase in the amplitude, the waves assuming a more regular shape of 15—20 cycles/sec. (Fig. 3, B).

2) Relationship between Harmine Tremor and Motor Actions of Other Drugs

The tremor-producing action of harmine is markedly influenced by a number of substances which excite or paralyse the central nervous system.

The synergistic action of a central excitant poison is more marked in the case of stimulant amines, such as methamphetamine. A subcutaneous injection of 0.03 mg./10 g. of methamphetamine in the mouse is sufficient to produce intense increase of movement and there is no appearance of a movement similar to tremor when administered alone. In such a case, curves recorded by the tracing method No. 1 only show numerous gentle waves of large amplitude, arising from walking and other gross motions. Simultaneous administration of harmine, in a tremor dose of 0.01—0.1 mg./10 g., and methamphetamine, in a dose of 0.003—0.03 mg./10 g., results in appearance of a pronounced tremor, which continues for 30 minutes or longer, with an amplitude several times larger than that produced by the sole administration of 0.1 mg./10g. of harmine (Fig. 4, A). The synergistic action of caffeine is similar to that of amphetamine but far weaker, administration of 5—10 mg./10 g. of caffeine resulting in a slight increase of the tremor. Synergistic action of convulsant poisons, such as picrotoxin, cardiazole, and strychnine, are weaker than of methamphetamine, the tremor produced by harmine being increased by the use of one-half the convulsive dose. These poisons frequently cause tetanic or clonic convulsions at the same time. Nicotine and eserine produce tremor when given alone but their synergistic action with harmine is not clear. Brucine, which also produce tremor by itself, slightly increases the tremor-producing action of harmine.

FIG. 4 A. Tracing of harmine movement in a mouse previously treated with methamphetamine (methylpropamine) (by Method No. 1)

FIG. 4 B. Tracing of harmine movement in a mouse previously treated with bulbocapnine (by Method No. 1)

The drugs that are more or less antagonistic against the tremor-producing action of harmine are bulbocapnine, methadione, sodium diphenylhydantoin, barbiturates, mephensine, and urethane, of which bulbocapnine and sodium diphenylhydantoin show distinct antagonistic actions. In a mouse which is manifesting the so-called bulbocapnine posture by the subcutaneous injection of 0.1 mg./10 g. of bulbocapnine, the administration of 0.1 mg./10 g. of harmine produces only a slight tremor, or none at all (Fig. 4, B). Sodium diphenylhydantoin also causes an increase in movements at 1 mg./10 g., and considerably inhibits the appearance of tremor by 0.1 mg./10 g. of harmine.

3) *Appearance of Harmine Tremor in Relation of Exision of Various Sections of the Brain*

Various sections of the mouse brain were coagulated by means of high frequency electric current or destroyed or excised with a fine scalpel and effects of these operations on harmine tremor were observed. It was thereby ascertained that destruction of the cerebral cortex and striatum caused distinct influence of the appearance of the tremor.

Tests were carried out by the total or partial excision of the central, outer, and front sections of the cerebral cortex. Appearance of harmine tremor is completely

FIG. 5 A. Tracing of the movement in a mouse with the cerebral cortex destroyed (by Method No. 1)

FIG. 5 B. Tracing of the harmine movement in a mouse with the striatum destroyed (by Method No. 1)

inhibited when the cortex is removed entirely, and such inhibition becomes less marked by the excision of the central section, and still less so by the removal of the outer sections. When the excision of the cerebral cortex is almost complete, tremor produced by harmine does not appear for three to five days (Fig. 5, A). If same small portion remains, tremor appears more or less within a few days in the majority of cases.

When the striatum is destroyed or excised, the harmine tremor is clearly inhibited if the part removed is large (Fig. 5, B). Inhibition of the appearance of tremor is more or less distinct when the posterior half is destroyed but there seemed to be no great difference in such inhibition whether the anterior or posterior half is destroyed.

When incision is made at the level of the midbrain, considerable influence is seen when the scalpel reaches a certain depth. If the incision reaches the tegmentum of the midbrain, on either side, the animal takes a peculiar posture and changes of movement and, at the same time, it becomes distinctly difficult to produce tremor by the administration of harmine. Removal or destruction of the hippocampus and thalamus exerts no appreciable influence on the appearance of the tremor. Harmine tremor fails to appear where the destruction of the thalamus has been made deeper, and the hypothalamus and subthalamus have been damaged.

In the case of the destruction or excision of the cerebellum, the impairment of the sense of equilibrium in the test animal makes it impossible to make an accurate observation but it may be assumed that some influence is caused on the appearance of the tremor.

From the results of the foregoing experiments, the appearance of the action of harmine seems to be distinctly inhibited by the destruction or excision of the cerebral cortex, striatum, and midbrain. The effect is most marked when the cortex is removed and this agrees, on the whole, with the points of attack of the extrapyramidal poisons reported by Tsukada(9) as being the cerebral cortex and striatum. The fact that the tremor-producing action of harmine can hardly be inhibited when part of the cerebral cortex remains is entirely in agreement with his report.

Final results have not been obtained, as yet, regarding the effect of a partial destruction of the subcortical nuclei.

SUMMARY AND CONCLUSIONS

The foregoing experimental results may be summarized as follows:

1. Detailed observations and analysis were made of the tremor, which is the specific action of extrapyramidal poisons, by means of three newly devised tracing methods.

2. The tremor produced by the administration of harmine is the most typical and its cycle is 15—20 per second.

3. The tremor produced by harmine is distinctly inhibited by bulbo-capnine.

4. The tremor produced by harmine is markedly increased by methamphetamine.

5. The tremor produced by harmine is inhibited by the primary destruction of various sections of the brain, especially by that of the cerebral cortex and striatum.

In short, harmine has the most powerful and specific tremor-producing action among the extrapyramidal poisons and it became clear that the cerebral cortex and striatum play an important part in its appearance. Moreover, this tremor-producing action is effectively inhibited by another extrapyramidal poison, such as bulbo-capnine.

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