DEPARTMENT OF PHARMACOLOGY, THE UNIVERSITY OF ROCHESTER, ROCHESTER, NEW YORK, U.S.A.

EFFECTS OF A TETRAHYDROCANNABINOL DERIVATIVE ON SOME MOTOR SYSTEMS IN THE CAT (1)

BY

E. S. BOYD AND D. A. MERITT

(Received for publication 9-1-1964*).*

The dimethylheptyl substituted tetrahydrocannabinol, DMHP, has been shown to depress spinal and brain stem polysynaptic reflexes (2) at a dose level of 0.1 mg/kg. Usually it does not affect the monosynaptic knee jerk significantly although in an occasional animal it will produce as much as a 50 % inhibition. DAGIRMANJIAN and BOYD (2) reported that both high and low spinal sections blocked the action of the drug on the polysynaptic flexion reflex, whereas a midbrain section did not. This indicated that the site of action was in the lower brain stem. In order to further localize the site of action, experiments have been performed both repeating the low spinal section and determining the effects of DMHP on reflexes which were either facilitated or inhibited by brain stem, cerebellar and forebrain stimulation. The effect of DMHP on hind limb flexion induced by forebrain stimulation was also studied.

MATERIALS AND METHODS

The experiments were carried out in cats of both sexes anesthetized with 15 mg/kg of pentobarbital supplemented with 15–20 mg/kg of chloralose. Low spinal sections, where performed, were made at the level of the thirteenth cervical vertebra. Two hours elapsed between performing the sections and further experimentation. The monosynaptic knee jerk, or myotatic reflex, was recorded in all experiments except for those concerned with flexion induced by forebrain stimulation. In addition, in most cats either or both of the polysynaptic reflexes, flexion and linguomandibular, were also recorded. All motor activity was recorded on smoked paper. The reflexes were induced by methods pre-

(1) This work was supported by U.S. Public Health Service grant MH 04230-03.

viously described (2). Rectal temperature was maintained between 34° and 38° C by means of a heated table. Rectal temperatures are indicated on all records.

For stimulation and recording two-wire electrodes were placed stereotaxically. These were similar to those described by STARK *et al.* (12) except that both wires projected 1.5 mm below the central strut. The atlas of SNIDER and NIEMER (11) was used as a guide. The electrode pair was used either as a bipolar electrode or as two monopolar electrodes with the stereotaxic instrument serving as an indifferent electrode. Sixty second trains of biphasic square-wave stimuli of 100 c.p.s., 1 msec duration and 3 to 8 volts were used for inducing facilitation or inhibition of reflexes due to brain stem and cerebellar stimulation. All electrode placements were confirmed by passing a d.c. current through the electrode and fixing the brain in formalin containing ferrocyanide. Frozen sections of 40 to 80 micron thickness were examined by the method of GUZMAN *et al.* (3).

A flexion of the contralateral hind limb involving a contraction of the anterior tibialis and associated muscles was produced by stimulation of the head of the caudate nucleus or of the adjacent internal capsule, at the level of the anterior commissure. The stimulus was a 30 second train of biphasic square-waves of 100 c.p.s., 2 msec duration and 4 to 8 volts. The flexion thus induced lasted several seconds, but in most cases it did not last as long as the stimulus. Effects on the linguomandibular reflex were also measured in some of these experiments, using the same stimulus parameters.

DMHP as a colloidal suspension in Tween 80 — normal saline (2) was given intravenously in a dose of 0.1 mg/kg. Thiopental was used in some experiments so that the action of DMHP could be compared with that of a barbiturate. The thiopental, dissolved in normal saline, was given intravenously in doses of 1 or 2 mg/kg. All injections were controlled by a prior injection of an equal volume of the vehicle. Blood pressure was recorded in all experiments by means of a mercury manometer attached to a cannula in the carotid artery. DMHP was given only once in each experiment because of its long duration of action.

RESULTS

Low Spinal Sections.

DMHP was given to 8 cats after low spinal section. The typical depression of the polysynaptic flexion reflex caused by DMHP was seen in 5 of these animals (Cat R-14, FIG. 1). In the other 3 there was either no depression or a slight facilitation of the reflex (Cat R-17, FIG. 1). A depression of the linguomandibular reflex was seen with the drug in all cases (FIG. 1). In addition, thiopental caused a depression of both the flexion and the linguomandibular reflexes in the 4 animals to which it was given.

Stimulation of the Brain Stem Reticular Formation.

Stimulation of the brain stem reticular formation was carried out in 30 cats. There was only a poor correlation between the areas stimulated and the effects produced. In 25 cats the electrode was between anterior 5 and posterior 2 (the interaural plane being 0) and 2 to 4.5 mm lateral. These placements were checked and were in the region of the brain stem facilitory area (9). In most of these placements facilitation was seen, but

in 2 there was inhibition of the flexion reflex and in 2 there was partial inhibition of the linguomandibular reflex, not related to a tonic closure of the jaw. In 5 cats the electrode was between posterior 9 and posterior 12, being lateral 2 or 2.5 mm. These placements were in the region of

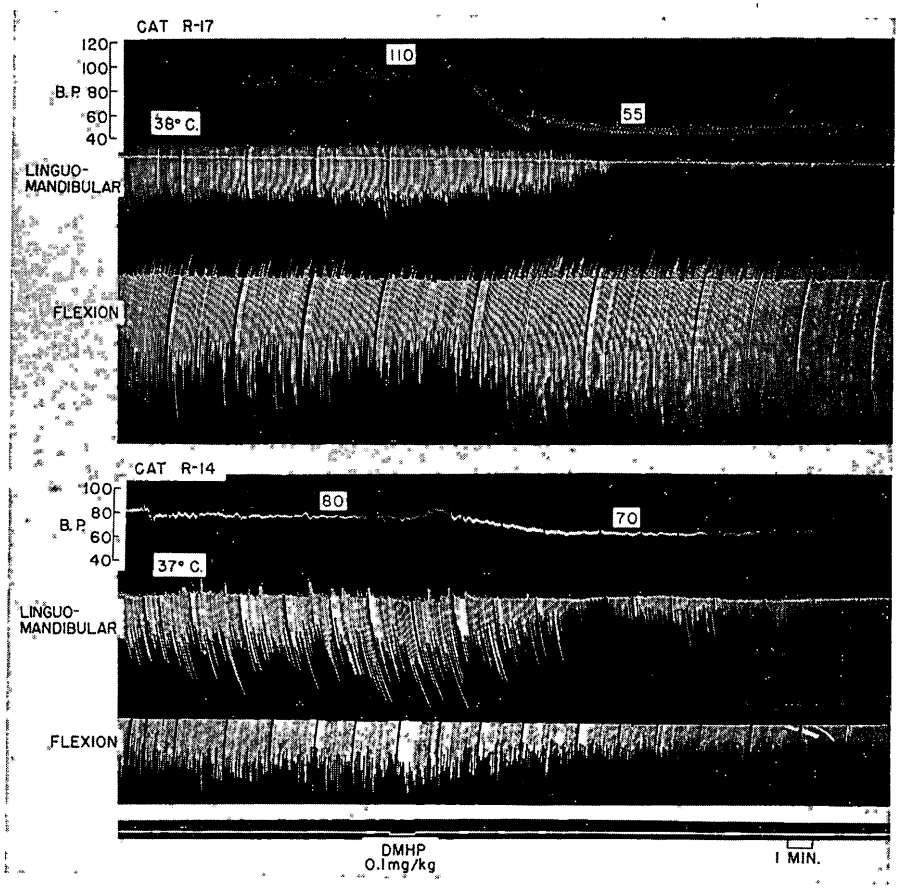

FIG. 1

The effects of 0.1 mg/kg of DMHP on blood pressure and flexion and linguomandibular reflexes in cats R-17 and R-14, following low spinal sections. Note that whereas the drug depressed the linguomandibular reflex in both animals, it depressed the flexion reflex only in cat R-14. The marker at the bottom shows the time of injection in both animals.

the bulbar inhibitory system of the reticular formation (6), but 3 cats showed facilitation of the flexion reflex; 2 showed facilitation of knee jerk; and one showed facilitation of the linguomandibular reflex. In several experiments, such as that illustrated in Fig. 5, brain stem stimu-

lation resulted in the simultaneous facilitation of one reflex and depression of another. In addition, there was no correlation between electrode placement and effects of DMHP. Therefore the results are presented without reference to electrode placement.

1. *Knee Jerk.* The knee jerk was facilitated in 20 cats. In 11 of these cats the drug had no effect on the facilitation. In one of them (FIG. 2) DMHP reduced the non-facilitated knee jerk by 30 per cent (an atypical action of DMHP) but the facilitated knee jerk remained at the same amplitude as before the drug. In the other 9 cats the facilitation of the knee jerk was depressed or eliminated by DMHP (FIG. 3, 4 and 5).

The knee jerk was inhibited in 4 cats. DMHP had no effect.

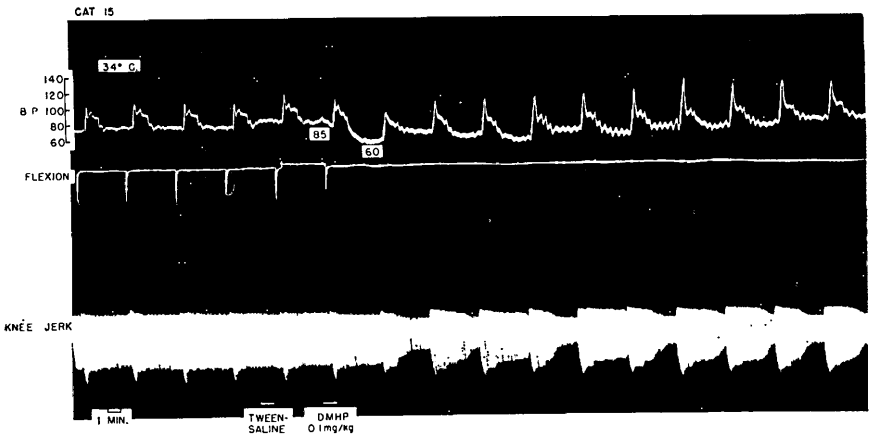

FIG. 2

The effects of 0.1 mg/kg of DMHP and the vehicle (Tween-saline) on blood pressure, flexion reflex and knee jerk in cat #15. The 60-second periods of stimulation are unmarked but are easily seen from the sharp rises in blood pressure. Electrode placement: anterior 1; lateral 4.5; vertical −2.

2. *Flexion Reflex.* The flexion reflex was facilitated in 10 cats. In several of these animals no flexion reflex was elicited at the stimulus voltage used except during the period of brain stem stimulation. In 6 of the animals, such as #15, which showed flexion only during the brain stem stimulation (FIG. 2), the drug partially or completely blocked the facilitated flexion. In the other 4 animals the drug did not depress the facilitated flexion reflex although it depressed the reflex in the intervals between periods of brain stem stimulation.

The flexion reflex was inhibited in 3 cats. In all of these the drug had no effect except to produce an overall depression of the reflex.

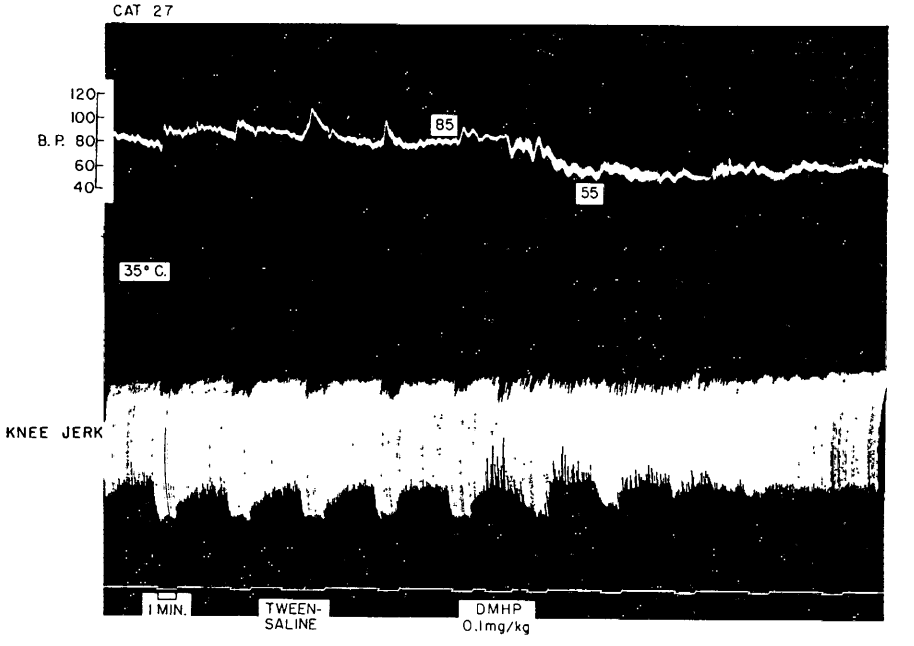

FIG. 3

The effects of 0.1 mg/kg of DMHP and of the vehicle (Tween-saline) on blood pressure and knee jerk in cat #27 during intermittent brain stem stimulation at: anterior 3; lateral 3; vertical −4. The bottom line shows the periods of stimulation and of drug administration.

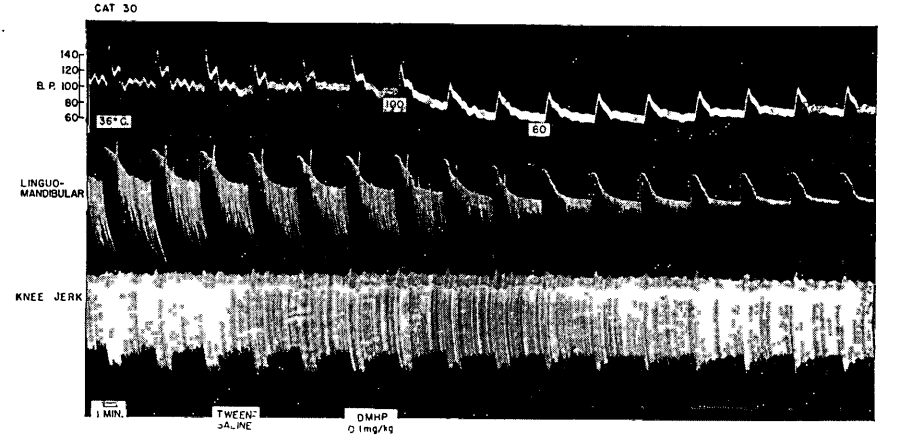

FIG. 4

The effects of 0.1 mg/kg of DMHP and of the vehicle (Tween-saline) on blood pressure, linguomandibular reflex and knee jerk in cat # 30 during intermittent brain stem stimulation at: anterior 1; lateral 3; vertical 0. The bottom line shows the periods of stimulation and of drug administration.

3. *Linguomandibular Reflex.* The linguomandibular reflex was not facilitated in any of the animals.

The linguomandibular reflex was inhibited in 18 animals. In 16 of these the inhibition was due to a tonic closure of the jaw during stimulation, as in #30 (FIG. 4). In all cases DMHP depressed the reflex in the periods between brain stem stimulation. In the other 2 cats, such as #34 (FIG. 5), the reflex was partially inhibited without tonic closure of the jaw. In both of these animals the drug depressed the reflex between periods of brain stem stimulation to a greater extent than it depressed the inhibited reflex.

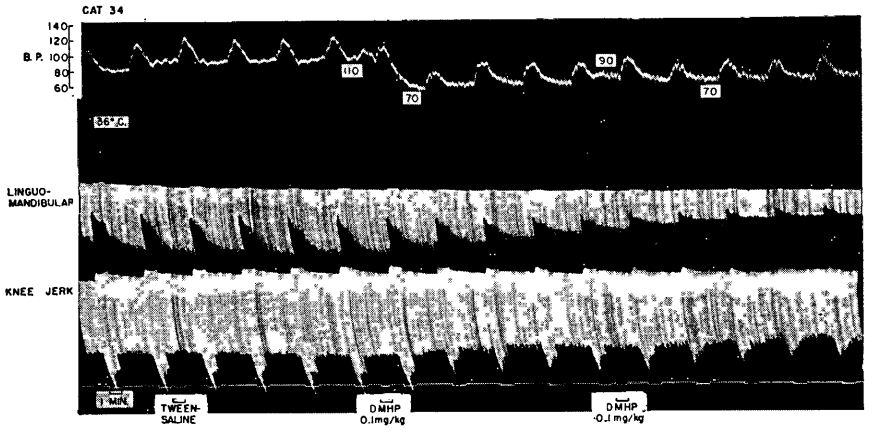

FIG. 5

The effects of 0.1 mg/kg of DMHP and of the vehicle (Tween-saline) on blood pressure, linguomandibular reflex and knee jerk in cat #34 during intermittent brain stem stimulation at: anterior 4; lateral 3; vertical −2. The bottom line shows the periods of stimulation and of drug injection.

Stimulation of the Cerebellum.

In 4 cats the stimulating electrode was placed in the fastigial nucleus which has been demonstrated to produce inhibition of motor systems (5). Stimulation by means of these electrodes caused a partial inhibition of the knee jerk in all 4 cats. DMHP had no effect on the knee jerk or on this partial inhibition of it. The flexion and linguomandibular reflexes were not measured.

Stimulation of Caudate Nucleus and Internal Capsule.

The region of the head of the caudate nucleus and the internal capsule were stimulated to produce a hindlimb flexion described below. In 21 of these animals the linguomandibular reflex was also measured. Knee

jerk and the flexion reflex were not measured because the induced flexion interferred with the measurement of hindlimb reflexes.

The linguomandibular reflex was facilitated by forebrain stimulation in 19 cats. The points of stimulation are shown by the solid circles in FIG. 6B. DMHP was given to 12 of these animals and in all cases it depressed the reflex between periods of forebrain stimulation. It also depressed the facilitated reflex, sometimes to a greater extent than the baseline reflex, as in cat #246 (FIG. 7), and sometimes to a lesser extent than the baseline reflex, as in cat #301 (FIG. 7). (The points of stimulation in these 2 animals are marked in FIG. 6B.) Thiopental, in doses of 2 mg/kg, was given to 3 of the animals. In one it had no effect. In the other 2 its action resembled that of DMHP.

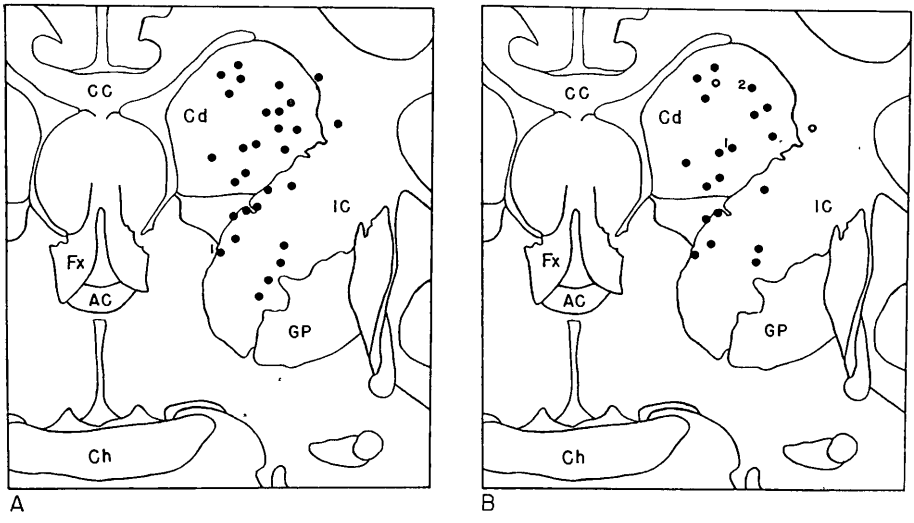

FIG. 6

Diagrams of the central region of the cat's brain at the level of the anterior commissure. A. Points where stimulation yielded flexion of the contralateral hind limb. The point marked (1) corresponds to cat #232 shown in Fig. 7. B. Solid circles are points where stimulation yielded facilitation of the linguomandibular reflex. Open circles are points where stimulation yielded inhibition of the linguomandibular reflex. The points marked (1) and (2) correspond to cats #246 and 301, respectively, shown in Fig. 7.

The linguomandibular reflex was inhibited by forebrain stimulation in 2 cats. The points of stimulation are shown by the open circles in Fig. 6B. DMHP and thiopental, 2 mg/kg, were tested in these animals. Both drugs depressed the reflex between periods of forebrain stimulation, but did not affect the size of the inhibited reflex.

Flexion Induced by Forebrain Stimulation.

As previously mentioned, in 29 cats a flexion of the contralateral hind limb was produced by electrical stimulation of the head of the caudate nucleus or of the adjacent internal capsule at the level of the anterior commissure. Points whose stimulation produced this response are shown in Fig. 6A. They were small enough so that when an area was

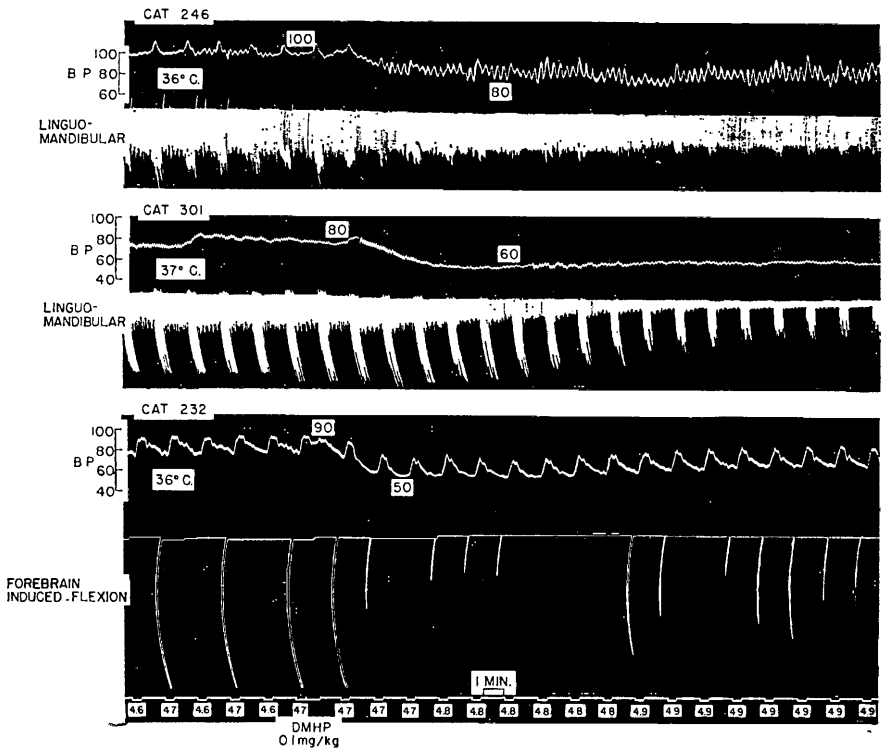

FIG. 7

The effects of 0.1 mg/kg of DMHP on blood pressure and the linguomandibular reflex in two cats and on blood pressure and forebrain induced flexion in another cat. The bottom line indicates periods of stimulation and drug injection in all three animals. In cat #246 the stimulus voltage was 7.8. In cat #301 it was 6.4. In cat #232 it was varied as marked.

found it was usually lost by moving the electrode 1 mm in any plane. For this reason it appears that the system producing this response extends into the caudate nucleus and that responses from the caudate nucleus were not simply due to spread of current into the internal capsule. In 4 animals all of the brain rostral to a plane 3 mm anterior

of the stimulating electrode was removed. This operation had no effect on the response. In 3 animals a section of the medulla just rostral to the decussation of the pyramidal tracts was made, starting dorsally and extending down to, but not injuring, the pyramidal tracts. This operation had no effect on the response. In 3 animals sections were made at the same level but starting ventrally with the homolateral pyramidal tract, then extending to the contralateral pyramidal tract and then dorsally up through the medulla. The sections were made by suction with a fine pipette and were extended slowly until the threshold for the response had been raised from its normal level of 4 to 8 volts to over 80 volts. The size of the lesion common to all 3 animals involved only the homolateral pyramidal tract and about one mm of tissue dorsal to it. In addition, in 2 other animals the electrical activity due to the stimulus was monitored throughout the medulla at the same level as that at which the sections were made. The response to the stimulus was found mainly in the medial part of the homolateral pyramidal tract. A second area of lesser response (smaller spike height) was found half way through the homolateral medulla, 0.5 mm from the midline.

DMHP was given to 14 cats with induced hindlimb flexion. Eight of these cats also received thiopental at a dose of 1 mg/kg. Both drugs uniformly depressed the induced flexion. An example of the effect of DMHP is shown in cat #232 in Fig. 7. The drug raised the threshold for the response in this case from 4.7 to 4.9 volts. The point of stimulation in this animal is marked in Fig. 6A. In 14 animals the average threshold stimulus intensity was 4.4 volts. DMHP raised this threshold to 5.4 volts with a standard error of 0.2. Thiopental raised the threshold to 5.2 volts with a standard error of 0.2. There was no statistically significant difference in the potency of these two drugs on this effect at the dose levels used.

Discussion

DMHP at doses of 0.1 mg/kg consistently depressed the polysynaptic flexion and linguomandibular reflexes, and occasionally even depressed the monosynaptic myotatic reflex. The experiments involving low spinal sections indicate that the drug has a marked but inconsistent depressant action on motor systems of the lower spinal cord. This resembled the action of the barbiturate, thiopental, except that the effect of the latter was seen consistently. The effects of DMPH on motor inhibitory and facilitory systems of the brain stem, the cerebellum and the caudate nucleus were also somewhat variable. For the most part the drug appeared

to have no significant action on the inhibitory systems. Although its action was variable on facilitory systems, the major effect was one of depressing such systems. Facilitation of the knee jerk due to brain stem stimulation was depressed in 9 of 20 animals. Facilitation of the flexion reflex due to brain stem stimulation was depressed in 6 of 10 animals. And facilitation of the linguomandibular reflexes due to forebrain stimulation was depressed in 12 of 12 animals. However, in the case of the flexion and linguomandibular reflexes the results were frequently not as easy to interpret as in the case of the knee jerk because with the polysynaptic reflexes both the reflex and its facilitation tended to be depressed.

The production of motor facilitation by stimulation of the head of the caudate was rather surprising since this region is widely accepted as belonging to an inhibitory system (5, 10). However, facilitation of motor activity by stimulation of the caudate nucleus is indicated by the work of METTLER and METTLER (7). PEACOCK and HODES (8) found that stimulation of many areas of the forebrain produced facilitation of motor activity. These areas included the caudate nucleus and the internal capsule at the same level as that studied in this work. In their experiments stimulation of these areas caused facilitation of cortically induced forelimb flexion, but not of the knee jerk. HODES *et al.* (4) also found that stimulation of various forebrain regions including the caudate nucleus and internal capsule at this same level could produce inhibition of both cortically induced forelimb flexion and knee jerk. This is in agreement with the inhibition of the linguomandibular reflex found in two of the experiments reported here.

The finding of hindlimb flexion due to stimulation of the caudate nucleus and the internal capsule was also surprising. Most sources state that stimulation of the caudate nucleus does not produce motor activity (10) and it is possible that all the effects seen in this work were due to spread of current to the internal capsule. However, the fact that some of the points in the caudate nucleus were as much as 3 mm from the internal capsule together with the fact that when a point yielding the response was found the electrode could be moved less than one mm in any plane without losing the response argues against this. It is also noteworthy that one of the illustrations in the paper by HODES *et al.* (4) shows the induction of a forelimb flexion by stimulation of a point approximately in the center of the caudate nucleus at this level.

Flexion due to stimulation of the caudate nucleus or of the internal capsule and facilitation of the linguomandibular reflex caused by stimulation at the same rosteral level were uniformly depressed by

DMHP. In addition, DMHP consistently depressed the polysynaptic flexion and linguomandibular reflexes. Since the depressant action of DMHP both in the spinal cord and on the facilitory system of the brain stem is variable, the consistent depression of polysynaptic reflexes by the drug must be explained in one of two different ways. In any animal it is likely that the drug will act at one or more of the levels at which its depressant action can occur, thus giving a consistent end result. On the other hand, the drug appears to have a consistent action at the level of the caudate nucleus and it is possible that it has a consistent depressant action on one or more of the other forebrain regions, such as the cingulate gyrus, septal nuclei, etc., found by PEACOCK and HODES (8) to produce facilitation of motor activity, but not investigated in the work reported here.

The depressant action of DMHP on motor systems resembles that of the barbiturate, thiopental, except that DMHP is about 10 times as potent as thiopental, and the action of thiopental is less variable. DMHP and a barbiturate have also been shown to have very similar actions on behavioral patterns in rats evoked by various schedules of free operant behavior (1).

SUMMARY

The tetrahydrocannabinol derivative, DMHP, consistently depressed the polysynaptic flexion and linguomandibular reflexes and occasionally depressed the monosynaptic knee jerk of the cat. It had a variable depressant action on the lower spinal cord and on the brain stem facilitory system. It had a consistent depressant effect on motor facilitation and on flexion induced by stimulation of the head of the caudate nucleus and the adjacent internal capsule. Its consistent depressant action on polysynaptic reflexes in the intact animal therefore appears to be due either to a summation of possible depressant actions along the neuraxis from the reflex system itself to the forebrain or to a consistent depressant action at one or more facilitory regions at the level of the forebrain.

REFERENCES

1. — BOYD, E. S., HUTCHINSON, E. D., GARDNER, L. C. and MERITT, D. A. *Arch. int. Pharmacodyn.*, 1963, *144*, 533.
2. — DAGIRMANJIAN, R. and BOYD, E. S. *J. Pharmacol.*, 1962, *135*, 25.

3. — GUZMAN, C., ALCARAZ, M. and FERNANDEZ, A. *Bol. Inst. estud. med. Biol. mex.*, 1958, *26*, 29.
4. — HODES, R., PEACOCK, S. M. and HEATH, R. G. *J. comp. Neurol.*, 1951, *94*, 381.
5. — LINDSLEY, D. B., SCHREINER, L. H. and MAGOUN, H. W. *J. Neurophysiol.*, 1949, *12*, 197.
6. — MAGOUN, H. W. and RHINES, R. *J. Neurophysiol.*, 1946, *9*, 165.
7. — METTLER, F. A. and METTLER, C. C. *Amer. J. Physiol.*, 1941, *133*, 594.
8. — PEACOCK, S. M. and HODES, R. *J. comp. Neurol.*, 1951, *94*, 409.
9. — RHINES, E. and MAGOUN, H. W. *J. Neurophysiol.*, 1946, *9*, 219.
10. — RUCH, T. C. and FULTON, J. F. "Medical Physiology and Biophysics", W. B. Saunders Co., Philadelphia, 1960.
11. — SNIDER, R. S. and NIEMER, W. T. "A Stereotaxic Atlas of the Cat Brain", University of Chicago Press, 1961.
12. — STARK, P., FAZIO, G. and BOYD, E. S. *Amer. J. Physiol.*, 1962, *203*, 371.