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Effects of microinjection of ouabain into the hypothalamus in cats

B. N. BASU RAY, W. M. BOOKER, S. N. DUTTA AND S. N. PRADHAN

Department of Pharmacology, Howard University College of Medicine, Washington, D.C., U.S.A.

Summary

1. In cats anaesthetized with chloralose, repeated injections of 20 μg ouabain were made either into the cerebral ventricles, or into the ventromedial nucleus of the hypothalamus (VMH), or intravenously whilst the electrocardiogram, arterial blood pressure and respiration were recorded.

2. The injections produced cardiac arrhythmias preceded by sinus bradycardia, variable changes in arterial blood pressure and respiratory depression. Death occurred either from ventricular fibrillation or from cardiac arrest.

3. The arrhythmias which occurred after the injections into the cerebral ventricles were not peripheral effects produced after absorption of the ouabain into the blood stream, because with intravenous injections larger amounts were required to produce the arrhythmias and to cause death than with intraventricular injections. The arrhythmias which resulted in death were due to an action on the VMH. With microinjections of ouabain into this region of the brain death occurred earlier and after smaller doses than after intraventricular injections.

4. While sinus bradycardia was abolished by bilateral vagotomy other cardiac arrhythmias were prevented by acute cardiac sympathectomy and cervical cord transection. Thus both the sympathetic and parasympathetic nervous systems appear to be involved in the production of these arrhythmias.

5. Since some of the cardiac arrhythmias obtained with ouabain in anaesthetized cats resemble the cardiotoxic effects seen in clinical practice during treatment with digitalis glycosides it is concluded that these effects, too, are, at least in part, central in origin, caused by an action on the VMH and mediated mainly via the sympathetic nervous system.

Introduction

Haley & Weinberg (1955) and Weinberg & Haley (1955) injected small doses of strophanthin-K into the third ventricle of unanaesthetized dogs and found that they produced various forms of cardiac arrhythmias. They suggested that the arrhythmias which were abolished by pentobarbital and by ganglion blocking drugs were of central origin, because they were not produced when the same doses of strophanthin were injected intravenously. Cardiac arrhythmias were also observed by Melville & Shister (1957) with injections of digitalis glycosides into the lateral ventricles of unanaesthetized cats, and by Solti, Marton & Takacs (1959) in cross-circulation experiments on dogs when strophanthin together with ephedrine was

injected into the isolated head circulation; the arrhythmias occurred in the recipient dog. More recently, the development of arrhythmias following intravenous injections of ouabain in anaesthetized cats was shown to be associated with an increase of the spontaneous electrical activity in cardiac sympathetic, vagus and phrenic nerves (Gillis, 1969; Gillis, Levitt, Raines, Sohn & Standaert, 1969). It was suggested that the arrhythmias were in part at least the result of centrally induced excitation in the autonomic nervous system produced by ouabain.

The present study was undertaken to identify the central site or sites where ouabain acts when producing the cardiac arrhythmias. Accordingly, microinjections of ouabain solution were made into the ventromedial nucleus of the hypothalamus (VMH) which is known to have some regulatory influence on the cardiovascular system, and which because of its close proximity to the third ventricle is readily permeated by drugs administered into the cerebroventricles. The effects of these injections were compared with those produced by ouabain injected into the lateral ventricle and intravenously.

Methods

Experiments were carried out in 41 cats of either sex weighing 2–4 kg under chloralose anaesthesia (50–60 mg/kg i.v.) and the animals were allowed to breathe spontaneously through a tracheal cannula. Femoral arterial pressure, electrocardiogram (Lead II) and respiration were recorded continuously during the experiment. Microinjections of ouabain were made into the VMH or the lateral ventricle (LV) of either side through a cannula-electrode placed stereotaxically in the respective sites according to the techniques of Fletcher & Pradhan (1969). The coordinates for the VMH (anterior 12 mm, lateral 1–1·5 mm and vertical −5 mm) and for the lateral ventricle (anterior 12 mm, lateral 1–1·5 mm and vertical 5 mm) were taken from the atlas of Jasper & Ajmone-Marsan (1961). The cannula-electrode consisted of a 22 gauge needle which, insulated except at its tip, served as a stimulating electrode as well. When the tip was in the VMH, electrical stimulation through the cannula-electrode elicited a pressor response. The correct placement was subsequently verified by histological examination of serial sections of the brain perfused with formalin saline and stained with cresyl violet.

Attempts were made to modify the effects of ouabain injected into the VMH by 3 surgical procedures, which interfere with the innervation of the heart, viz., (1) bilateral vagotomy, (2) acute cardiac sympathectomy which consisted of bilateral removal of the stellate ganglia together with portions of the cervical sympathetic trunks and superior cervical ganglia (Nielsen & Owman, 1969), and (3) transection of the spinal cord at C_2 level.

Ouabain was dissolved in normal saline and the pH was adjusted to 7·4. Microinjections of ouabain (20 μg) were given in volumes of 0·01 ml into the VMH and of 0·01–0·02 ml into the LV at intervals of 20 min until death. For intravenous administration doses of 40 μg were repeated under similar schedules. The same volumes of normal saline were used as controls. The total cumulative doses of ouabain thus administered which caused death was designated as the lethal dose (LD) and the time to cause death was calculated from injection of the first dose. The data concerning certain parameters at a particular dose level were expressed as the mean and standard error of the mean (S.E.). Student's t test was performed to ascertain the level of significance of certain data.

Results

Effects with different routes of injection

Injections into the VMH

In 12 out of 15 cats in which ouabain was injected into the VMH, the first injection (20 μg) produced within 5 to 10 min a mean fall of arterial blood pressure of 12 ($\pm$2)%. In 3 cats the pressure was increased by 32 ($\pm$7)%. Subsequent injections of the same dose of ouabain produced changes which had no relation to the response produced by the first injection. For instance, after the second injection a fall (10$\pm$2%) in blood pressure occurred in only 4 of the 12 cats in which the first injection had produced a fall (9$\pm$3%) it rose in 5 cats by 37 ($\pm$9)% and produced no change in the other 6 cats. Of the 5 cats in which the second injection had produced a rise, the first injection had produced a rise in 2 and a fall in 3 cats.

In 10 of the 12 cats in which the first injection had produced a fall in blood pressure, the fall was associated with a reduction in heart rate of 30 ($\pm$5)%. In one cat in which blood pressure fell, the heart rate increased by 12% and in the other four cats it did not change. Thus the changes in heart rate corresponded to the blood pressure changes in 10 of the 15 cats. The changes in heart rate produced by subsequent injections again showed no relation to the response produced by the first dose. A second dose produced a reduction in heart rate of 25 ($\pm$6)% in 5 cats, an increase of 15 ($\pm$6)% in 4 cats, and no change in 6 cats.

In five cats in which a second injection produced a reduction in heart rate the first injection had produced a reduction in 3 and no change in 2 cats. While in 4 cats where the second injection produced an increase in the heart rate the first injection had produced reduction in 2 cats, increase in one and no change in the other. The six cats in which the second injection produced no change in heart rate had shown reduction in heart rate following the first injection.

All 15 cats showed several types of cardiac arrhythmias. In five cats the first injection produced sinus bradycardia, and signs of impaired A-V conduction, such as lengthening of the P-R and R-R intervals, first or second degree of A-V block, idioventricular rhythm and nodal rhythm; in addition, ventricular extrasystoles appeared. In the other cats, repeated injections of 20 μg were required to produce these ventricular arrhythmias. Sinus bradycardia and arrhythmias due to impaired conduction were usually associated with a fall while ventricular extrasystoles were often associated with a rise in blood pressure. Figure 1 illustrates the sequence in the development of arrhythmias after injection of cumulative doses of ouabain into the VMH in a cat, in which the first injection had produced only sinus bradycardia. Ventricular extrasystoles (trigeminy) and ventricular tachycardia developed after cumulative doses of 60 to 80 μg respectively; subsequent doses produced cardiac arrest and death.

Injections of ouabain into the VMH produced a decrease of respiratory amplitude in most of the cats. The respiratory rate was also decreased after the first injection and subsequently increased by repeated injections in the majority of the cats (Fig. 1).

Mydriasis was an extracardiac effect observed in 9 of the 15 cats and was often associated with the onset of cardiac effects. Other effects, viz., salivation, micturition, defaecation and jerky body movements occurred less frequently.

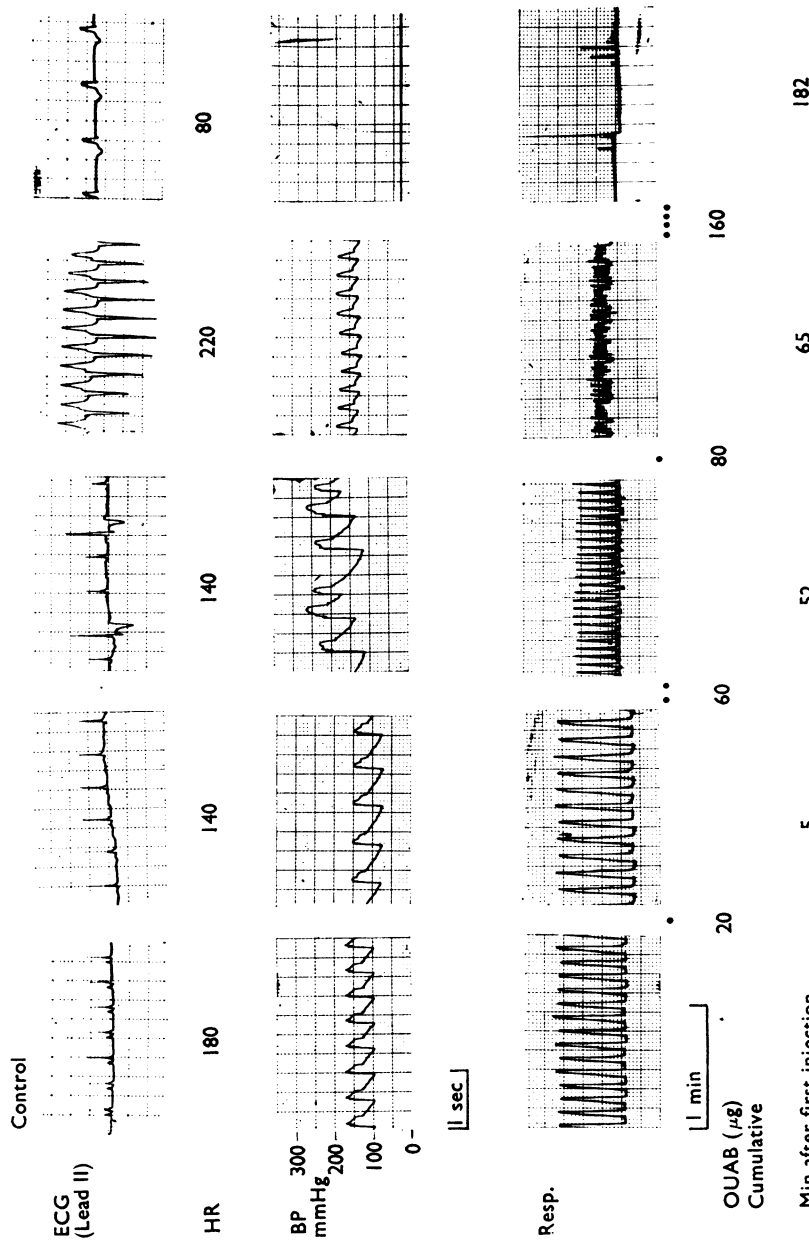

FIG. 1. Electrocardiogram (ECG) heart rate (HR), arterial blood pressure (BP) and respiration (Resp.) of a cat anaesthetized with chloralose. Each dot (.) represents an injection into the VMH of 20 μg ouabain. The injections were made at 20 min intervals until death.

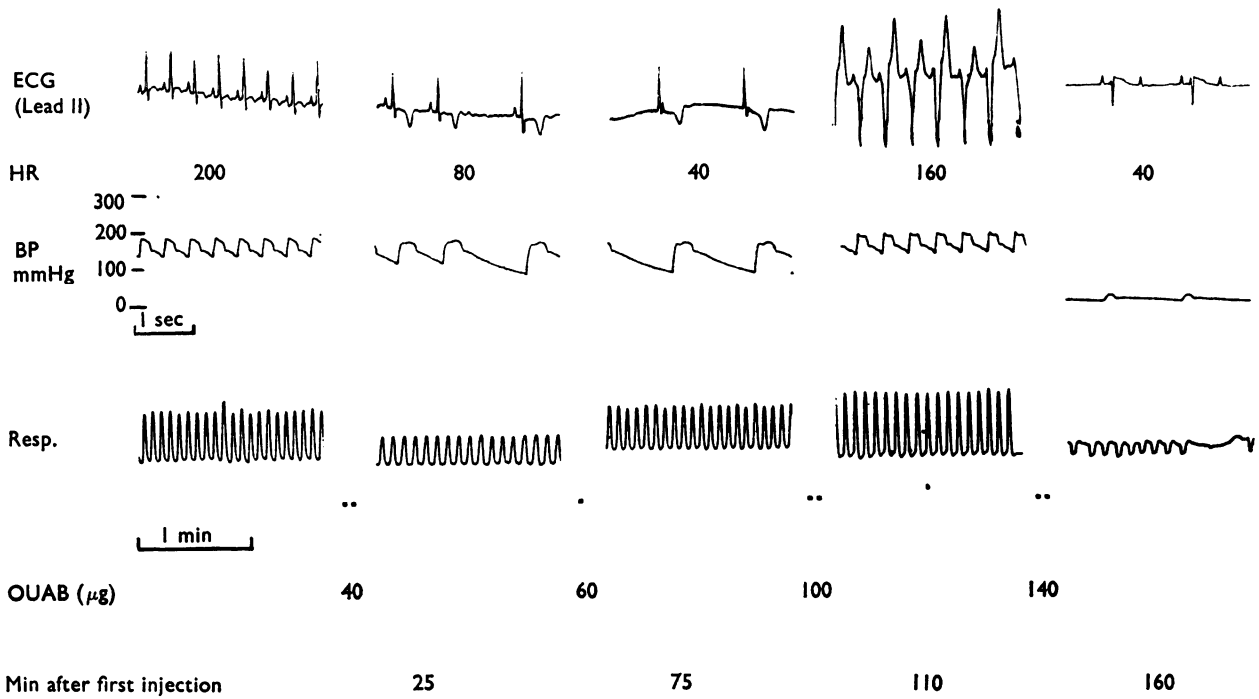

FIG. 2. Electrocardiogram (ECG) heart rate (HR), arterial blood pressure (BP) and respiration (Resp.) of a cat anaesthetized with chloralose. Each dot (.) represents an injection into the lateral ventricle of 20 μg of ouabain. The injections were made at 20 min intervals until death.

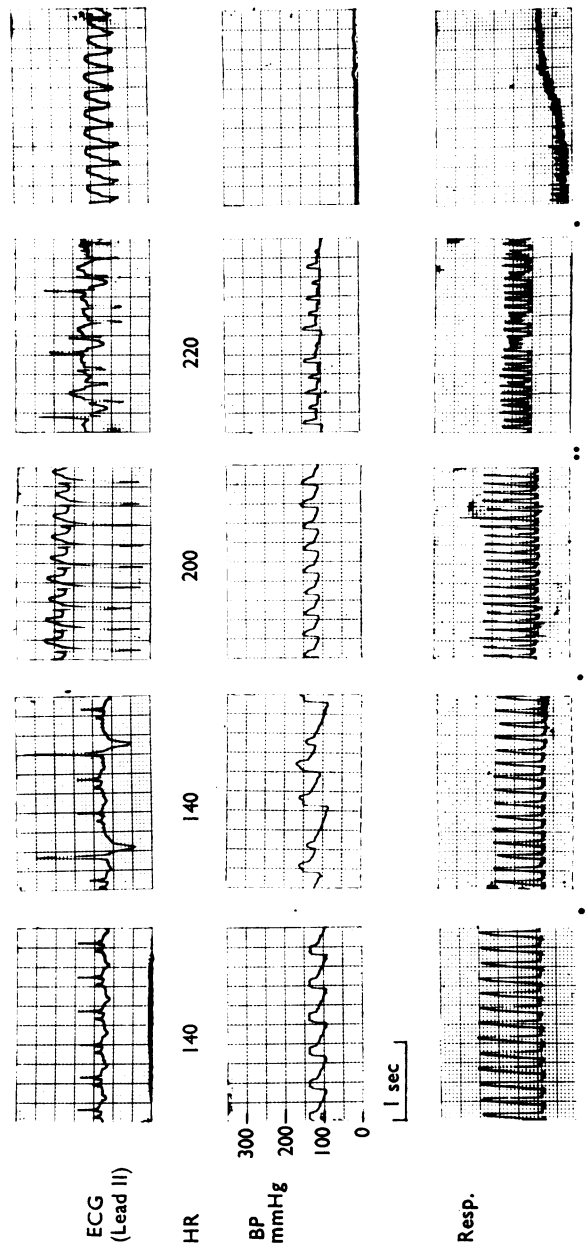

FIG. 3. Electrocardiogram (ECG), heart rate (HR), arterial blood pressure (BP) and respiration (Resp.) of a cat anaesthetized with chloralose. Each dot (.) represents an intravenous injection of 40 μg ouabain. The injections were made at 20 min intervals until death.

Injections into the lateral ventricle

In seven cats in which ouabain injections (20 μg) were made into the cerebral ventricles, the effects were similar to those described for the injections into the VMH. After the first injection, blood pressure fell by 15 ($\pm$6)% in five, and rose 32% in two cats, whereas heart rate decreased by 20 ($\pm$6)% in four, remained unchanged in one and increased by 15% in the remaining two cats. Subsequent injections resulted in similar variability in changes in blood pressure and heart rate.

Figure 2 illustrates some of the arrhythmias developed after injection of ouabain into the lateral ventricle of one cat. The first injection (20 μg) produced a fall in blood pressure and sinus bradycardia. After a cumulative dose of 40 μg there was further slowing of the heart rate and impairment of A-V conduction as seen by an increase in the R-R interval. Nodal rhythm and ventricular tachycardia developed after 60 and 100 μg respectively. Subsequent doses produced cardiac arrest and death. Effects of intraventricular injection of ouabain differed from that of intrahypothalamic injection in the absence of A-V dissociation and in the occurrence of ventricular fibrillation in three and cardiac arrest in four cats.

The respiratory and other extracardiac effects were the same as on injection into the VMH except that jerky forelimb movements were not observed.

Intravenous injections

In six cats the effects of repeated intravenous injection of 40 μg ouabain were examined. In all of them the first injection resulted in a rise of blood pressure of 25 ($\pm$5)% and a decrease in heart rate of 10 ($\pm$3)%, but arrhythmias developed in 2 cats only. The second injection produced a rise in blood pressure of 10 ($\pm$3)% in four cats, and a fall of 28% in the other two; arrhythmias appeared in all of them. Figure 3 shows the development of arrhythmias in one of these cats after intravenous injections. Injection of the first dose (40 μg) produced ventricular extrasystole (trigeminy) which was followed by ventricular tachycardia after 80 μg, multifocal ventricular extrasystole after 160 μg and ventricular fibrillation after injection of 200 μg of ouabain. Although in two cats ventricular arrhythmias developed after the first injection, larger doses were required in the other four animals. Ventricular fibrillation was the cause of death in all the animals, which differs from the result after injection into the VMH, or into the lateral ventricle, when cardiac arrest was more often the cause of death.

The first injection produced an initial reduction in amplitude, followed by an increase in both rate and amplitude of respiration after second injection. Subsequent injections produced shallow tachypnoea (Fig. 3).

Salivation occurred in two animals while micturition and defaecation were observed in the terminal stage in five of the six animals. Mydriasis and jerky body movements were not observed in these animals.

Lethal dose of ouabain and time to cause death

The results obtained with different routes of ouabain injections are summarized in Fig. 4. There was a significant reduction in the lethal dose and time to cause death after injections into the VMH, as compared with these values after intraventricular and intravenous injections, and the lethal dose after intraventricular injection was also significantly less than that after intravenous injection. However, the time to cause death after injections through these two routes was not significantly different.

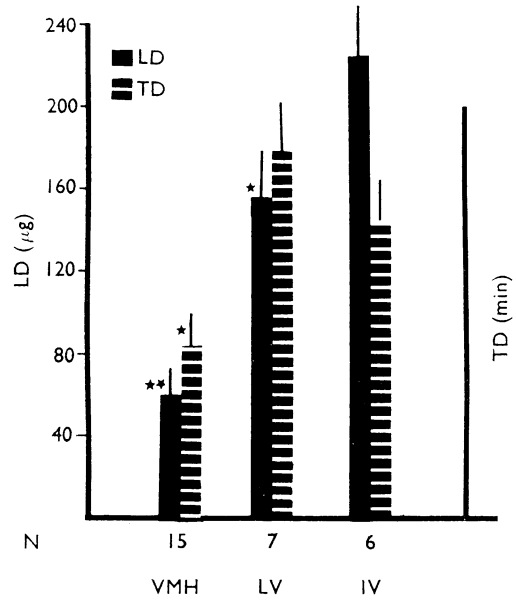

FIG. 4. Cumulative lethal doses (LD) and times to cause death (TD) in min after repeated injections of ouabain, at 20 min intervals until death, into anaesthetized cats. (VMH) injection into the VMH (20 μg each time); (LV) injection into the lateral ventricle (20 μg each time); (IV) intravenous injections (40 μg each time). N number of cats. $^{**}P<0.001$ compared to LV and IV injections. $^{*}P<0.05$ compared to IV injections. ■ LD. = TD.

Effects of surgical procedures on the responses to injections into the VMH

The results are summarized in Table 1 which gives the incidence of arrhythmias, the lethal doses of ouabain and the time to cause death.

Bilateral vagotomy performed in five cats at the onset of ouabain-induced sinus bradycardia abolished the bradycardia, but had no effect on the development of further arrhythmias; it also did not affect the lethal dose or the time to cause death.

Ouabain was administered after stabilization of blood pressure following acute cardiac sympathectomy in four cats. No arrhythmias appeared after the first two or three injections; therefore without altering the normal interval the dose was increased from 20 to 40 μg for each of the subsequent injections. Nevertheless no arrhythmias developed except in one cat, and in this the only form of arrhythmia was that of ventricular extrasystoles which appeared after the injection of 80 μg but disappeared after a short time in spite of further injection of another 40 μg dose. The lethal dose was significantly increased. The time to cause death was

TABLE 1. *Effects of surgical procedures on incidence of arrhythmias, lethal dose and time to cause death after intrahypothalamic injection of ouabain*

Surgical procedures	Incidence of arrhythmia	Lethal dose (μg)	Time to cause death (min)
Control	15/15	60±13	83±15
Bilateral vagotomy	5/5	64±20	69±24
Acute cardiac sympathectomy	1/4	115±17*	63±10
Spinal cord transection	1/4	—†	—†

*Significantly higher than the control, $P<0.05$.
†No death occurred after injection of cumulative doses up to 200 μg.

not significantly altered, but the comparison is not quite valid because the dose of ouabain was doubled after the second or third injection without altering the interval.

The cervical cord was transected in four cats immediately after the appearance of ouabain-induced arrhythmias. It resulted in an initial rise in blood pressure with accentuation of the arrhythmias followed by a fall in blood pressure and, in three of the four cats, by disappearance of the arrhythmias. Doses of ouabain up to 200 μg (larger doses were not given) were not lethal in any of the four cats.

Discussion

The cardiac arrhythmias produced by ouabain injected into the cerebral ventricles can be attributed to an action on the VMH, reached by penetration from the third ventricle, because ouabain produced the same kind of arrhythmias when injected into this region of the brain and with repetition of such injections the accumulated lethal dose was smaller than with intraventricular injections. The finding that on intravenous injections larger doses were required to produce the arrhythmias, and to cause death, than on injection into the VMH or even into the cerebral ventricles is in agreement with the conclusion that the arrhythmias are central in origin and are not a peripheral effect produced after absorption of the ouabain into the blood stream. The efferent nervous pathway by which the arrhythmias are produced is mainly the sympathetic, because the arrhythmias were found to be prevented by cardiac sympathectomy as well as by transection of the spinal cord, except in one cat which developed ventricular extrasystoles, but none of the other arrhythmias. Bilateral vagotomy, on the other hand, did not prevent the development of the arrhythmias; its only effect was an abolition of the initial sinus bradycardia. The ouabain-induced arrhythmias may therefore be initiated by parasympathetic overactivity but they are the result of sympathetic stimulation. The involvement of the sympathetic system in the effects of ouabain is also evident from the mydriasis produced by its injection into the VMH and the cerebral ventricles.

Cardiac arrhythmias similar to those produced by ouabain have been obtained with electrical stimulation of subcortical areas of the brain including the hypothalamus and have been described by a number of authors (Fuster & Weinberg, 1960; Weinberg & Fuster, 1960; Manning & Cotten, 1962; Melville, Blum, Shister & Silver, 1963; Hockman, Mauck & Hoff, 1966; 1967), and attributed also to a sympathetic discharge. On the other hand, Parker, Gunn & Lynn (1962) attributed the arrhythmias resulting from stimulation of the posterior hypothalamus to parasympathetic stimulation, and Manning & Cotten (1962) postulated involvement of both the sympathetic and parasympathetic nervous systems in the development of arrhythmias.

Respiratory depression produced by ouabain, as in the present experiments on anaesthetized cats, has also been obtained in rats, for instance by Bergman, Chaimovitz, Costin, Gutman & Ginath (1967) on implantation of ouabain (10–100 μg) into the hypothalamus of conscious rats and by Dal Ri & Schmidt (1960) after intracarotid injection of strophanthin-K in decerebrated rats. Prolonged apnoea with epileptiform discharge in the electroencephalogram was reported by Feuerstein, Manz, Kurtz, Isch-Treussard & Tempe (1968) in an infant during severe digitalis intoxication. Cameron (1967), on the other hand, obtained hyperventilation in

unanaesthetized rabbits on injection of ouabain into the cerebroventricles, and Sohn, Gillis, Raines & Levitt (1969) found in anaesthetized cats that ouabain given intravenously produced respiratory stimulation with increased electrical activity in the phrenic nerve. The conditions under which ouabain produced depression or stimulation of respiration are thus not clear.

Some of the arrhythmias produced by ouabain in the anaesthetized cat resemble the cardiotoxic effects that have been described in clinical practice during cardiac glycoside therapy. This suggests that they, too, are at least in part, central in origin, due to an action on the VMH and mediated mainly via the sympathetic nervous system.

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