Br. J. Pharmacol. (1993), **110**, 253–256

Centrally administered ouabain aggravates rapid-eye-movement-sleep-related bradyarrhythmias in freely moving rats

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1 The effects of continuous infusions of ouabain on bradyarrhythmias (cardiac pauses for 0.5 s or longer) during sleep were examined in freely moving Wistar-Kyoto rats.

2 In a control group ($n = 7$), saline was infused into both the lateral ventricle and the femoral vein. In an intracerebroventricular (i.c.v.) ouabain group ($n = 7$), ouabain was infused centrally, such that each rat received three stepped doses of 1, 10, and 100 ng kg^{-1} h^{-1} for 3 days at each dose, while saline was infused systemically. In an intravenous (i.v.) ouabain group ($n = 7$), ouabain was infused systemically at the same doses as the i.c.v. ouabain received, while the simultaneous i.c.v. infusion of saline was carried out.

3 Three-day i.c.v. infusions of the three stepped doses of ouabain caused a dose-dependent increase in the frequency of bradyarrhythmias during rapid-eye movement (REM) sleep without affecting the time spent in REM sleep, arterial pressure, average heart rate, or the frequency of bradyarrhythmias during non-REM sleep. Intravenous ouabain or i.c.v. saline had no effects on the frequency of bradyarrhythmias.

4 Intrinsic CNS activity during REM sleep may be involved in the centrally mediated arrhythmogenic properties of ouabain during sleep.

Keywords: Ouabain-induced arrhythmia; rapid-eye-movement sleep; central nervous system; limbic system; freely moving rat

Introduction

Digitalis-induced arrhythmias occur more often during sleep than during daytime (Otsuka, 1980). Their diurnal distribution does not correspond to the diurnal change in the serum concentrations of digitalis agents (Otsuka, 1980). The central nervous system (CNS) activity during sleep may be of significance in the arrhythmogenic properties of digitalis. The CNS-mediated effects of digitalis on cardiac rhythm have been examined extensively (Somberg & Smith, 1979; Gillis & Quest, 1980) but the high incidence of digitalis-induced arrhythmias during sleep cannot be explained by the findings obtained from earlier experimental studies in which anaesthetized animals or large doses of digitalis enough to induce seizures in conscious animals were used.

The objectives of this study were to examine the CNS-mediated effects of the digitalis agent, ouabain, on the cardiac rhythm during sleep in freely moving rats. In a preliminary study (Tadokoro *et al.*, 1991), we have confirmed that the chronic intracerebroventricular (i.c.v.) infusion of ouabain at a dose of 1 μg kg^{-1} h^{-1} induces epileptic discharges on the electroencephalogram (EEG) only during rapid-eye-movement (REM) sleep without arousing rats. We therefore selected smaller doses that do not result in any EEG abnormalities for the present study.

Methods

Surgical preparation

Twenty-one male Wistar-Kyoto rats from Charles River Japan, 12–14 weeks of age, weighing 280–290 g, were used. The care of the animals was in strict accordance with the guiding principles of the Physiological Society of Japan. The following electrodes and tubes were implanted under pentobarbitone anaesthesia (40 mg kg^{-1}, i.p.): for the monitoring of the EEG, two stainless-steel screws into the bilateral frontal bones; for recording the electrooculogram (EOG), two small loops of stainless-steel wire beneath the skin at the inner and outer canthi of one eye; for recording the electrocardiogram (ECG), two vinyl tubes containing saturated salt solution and 0.5% agar, plugged bipolarly with Ag-AgCl electrodes, under the skin at the right foreleg and the left hindleg; for measurement of arterial pressure, a Teflon tube into the abdominal aorta through the femoral artery; for the i.c.v. infusion of drugs, a 24-gauge, 20-mm-long stainless-steel tube into the lateral ventricle; for the intravenous (i.v.) infusion of drugs, a Teflon tube into the femoral vein. The leads and tubes from peripheral sites were tunnelled subcutaneously into an opening on the head. All the leads were soldered to the pins of a miniature male socket cemented on the skull. A female socket and miniature electrical swivel with three fluid channels was connected to the male socket and the three tubes. After surgery, the rat was transferred to a plastic box in an electrically shielded and soundproof room where fluorescent lights provided 100-lux illumination under a 14 h/10 h light-dark schedule. The temperature in the room was kept at 24 ± 1°C. The animals were allowed free access to normal rat chow (CRF-1, Charles River Japan) and distilled water, and were allowed to recover for 10 days before polygraphic recordings.

Infusion protocols

Twenty-one rats were divided into control ($n = 7$), i.c.v. ouabain ($n = 7$), and i.v. ouabain ($n = 7$) groups. The infusion study was started after the recovery period.

In the control group, saline was infused both intracerebroventricularly and intravenously for 15 days.

In the i.c.v. ouabain group, for the first 3 days saline was infused intracerebroventricularly; for the next 9 days ouabain dissolved in saline was infused intracerebroventricularly, such that each rat received three stepped doses of 1, 10, and

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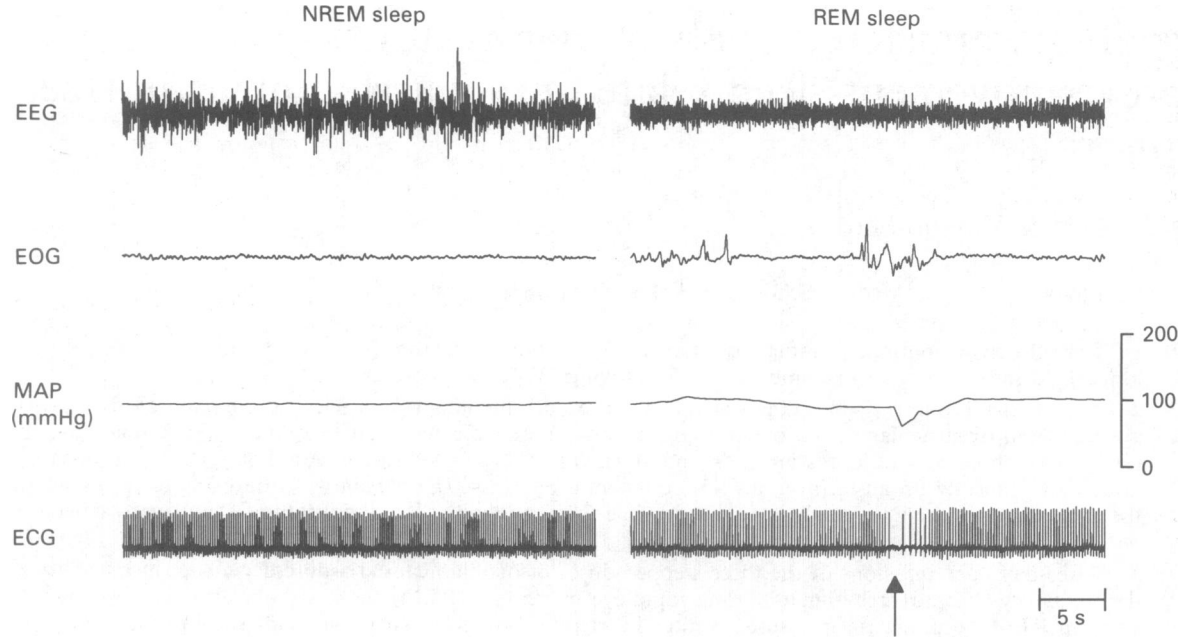

Figure 1 Typical polygraph recordings during non-rapid-eye-movement (NREM) sleep and REM sleep. Shown are the electroencephalogram (EEG), electrooculogram (EOG), mean arterial pressure (MAP), and electrocardiogram (ECG). An arrow indicates a bradyarrhythmia episode.

100 ng kg^{-1} h^{-1} for 3 days at each dose; for the next 3 days saline was infused intracerebroventricularly. For these 15 days, a simultaneous i.v. infusion of saline was carried out.

In the i.v. ouabain group, for the first 3 days saline was infused intravenously; for the next 9 days ouabain dissolved in saline was infused intravenously at the same doses as the i.c.v ouabain group received; for the next 3 days saline was infused intravenously. For these 15 days, a simultaneous i.c.v. infusion of saline was carried out.

All the infusions were at a flow rate of 1.6 μl h^{-1}, which is 1/75 of the rate of cerebrospinal fluid production in rats (Mann *et al.*, 1978). The positions of all the i.c.v. tubes were confirmed at *post-mortem* examination.

Polygraphic recordings

The EEG, EOG, mean arterial pressure (MAP), and ECG were recorded on polygraph paper and FM magnetic tape. The electrical signals of the arterial pressure were digitized through an analog-to-digital converter mounted in a personal computer, which automatically calculated and stored the 3-day averages of the MAP and heart rate (HR) every 3 days.

Sleep staging

According to our previous criteria (Saito *et al.*, 1983), sleep states were identified in 10 s epochs and were divided into the following three states from the EEG and EOG: wakefulness, non-REM (NREM) sleep, and REM sleep.

Definition of cardiac arrhythmias

The definition of tachyarrhythmias was the same as the criteria for man. Bradyarrhythmias were defined as cardiac pauses for 0.5 s or longer; as the normal HR of rats is 300–350 beats min^{-1} (Otsuka *et al.*, 1986), they are equivalent to cardiac arrests lasting 3 s or longer in man.

Statistical analysis

The physiological parameters and the frequency of cardiac arrhythmias were tested by a mixed model analysis of

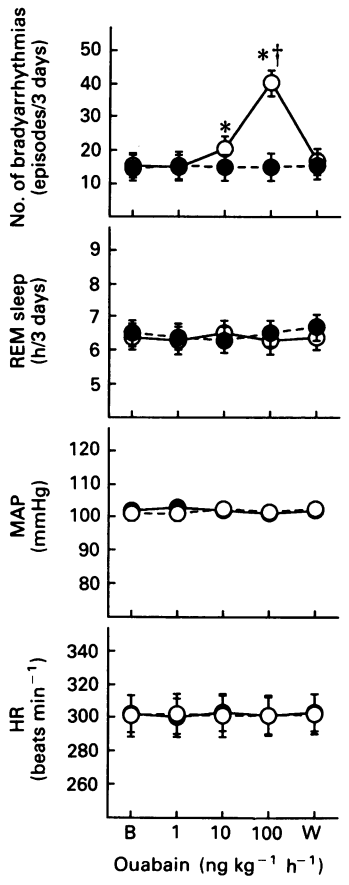

Figure 2 Effects of intracerebroventricular (○) or intravenous (●) ouabain infusion on the frequency of bradyarrhythmias during rapid-eye-movement (REM) sleep, on the time spent in REM sleep, on mean arterial pressure (MAP), and on heart rate (HR). Values are mean ± 95% simultaneous confidence intervals (overall $\alpha < 0.05$). *$P < 0.05$ from baseline period (B); †$P < 0.01$ from middle-dose infusion period (Tukey-studentized range method for a least significant difference test). W, withdrawal period.

variance. A 95% simultaneous confidence interval for the mean (overall $\alpha < 0.05$) was estimated from 5% point of the studentized form of the largest variance. A *post-hoc* analysis for multiple comparisons was performed by a Tukey studentized range method for a least significant difference test. Durations of cardiac pauses in bradyarrhythmia episodes were examined by a Kruskal-Wallis test, because a normal distribution of them could not be assumed. Differences were considered significant at $P < 0.05$.

Drugs

Ouabain was purchased from Sigma Chemical, St Louis, MO, U.S.A., and pentobarbitone sodium from Abbott Laboratories, IL, U.S.A.

Results

Examples of polygraph recordings are presented in Figure 1. No cardiac tachyarrhythmias were observed. In every rat, bradyarrhythmias such as sinus arrest, sinoatrial block, and type I (Wenckebach) second-degree atrioventricular block were found only during REM sleep.

In the control group, no time-dependent changes in the physiological parameters or the frequency of bradyarrhythmias were found during saline infusion into both the lateral ventricle and the femoral vein (data not shown). The effects of i.c.v. or i.v. ouabain infusion are summarized in Figure 2. Three-day i.c.v. infusions of the three stepped doses of ouabain caused a dose-dependent increase in the frequency of bradyarrhythmias during REM sleep without affecting the time spent in NREM or REM sleep, MAP, or HR. In the i.v. ouabain group, no effects of ouabain were found on the physiological parameters or the frequency of bradyarrhythmias. The frequency of bradyarrhythmias in the control group did not differ from baseline conditions in the two ouabain groups.

Types of bradyarrhythmias and durations of cardiac pauses in bradyarrhythmia episodes were analysed in the i.c.v ouabain group. Ouabain infusion had no effects on these characteristics of bradyarrhythmias (Figure 3).

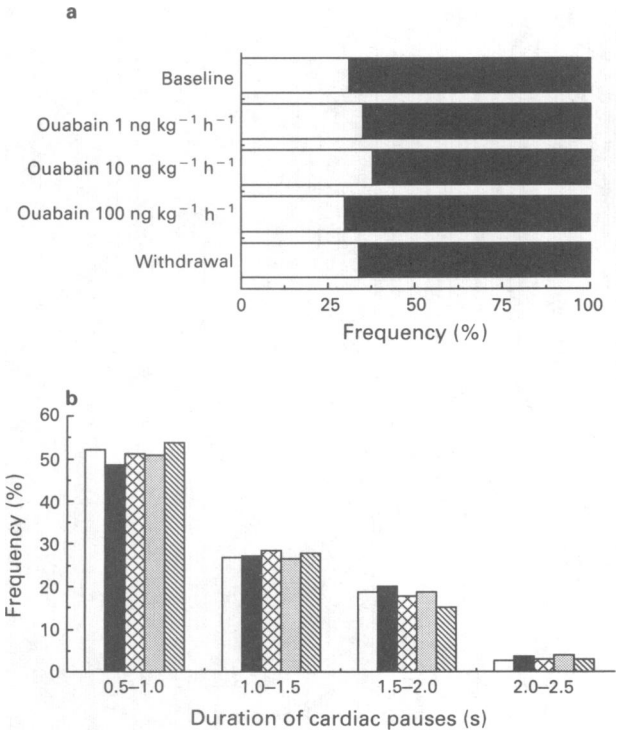

Figure 3 (a) Effects of intracerebroventricular ouabain infusion on the types of bradyarrhythmias. Open bars, sinus arrest and sinoatrial block; solid bars, type I (Wenckebach) atrioventricular block. (b) Effects of intracerebroventricular ouabain infusion on the frequency distribution of durations of cardiac pauses in bradyarrhythmia episodes. Open columns, baseline; solid columns, 1 ng kg^{-1} h^{-1} ouabain; cross-hatched columns, 10 ng kg^{-1} h^{-1} ouabain; stippled columns, 100 ng kg^{-1} h^{-1} ouabain; hatched columns, withdrawal.

Discussion

The actions of 'cardiac' glycosides, digitalis agents, on CNS functions have been studied for many years; they are also recognized as 'neural' glycosides (Gillis & Quest, 1980). Digitalis agents affect the release, uptake, synthesis, degradation, and storage of neurotransmitters such as noradrenaline, dopamine, 5-hydroxytryptamine, acetylcholine and γ-aminobutyric acid (Gillis & Quest, 1980). Digitalis-induced arrhythmias have been examined extensively; centrally administered digitalis induces tachyarrhythmias through sympathetic activation. However, the high incidence of digitalis-induced arrhythmias during sleep cannot be explained by the findings obtained from the earlier experimental studies in which anaesthetized animals or doses of digitalis large enough to induce seizures in conscious animals were used. Much attention must be given to whether the chronic i.c.v. infusion of a small dose that does not induce convulsions or EEG abnormalities produces arrhythmias. The present study shows that centrally administered ouabain induces bradyarrhythmias during REM sleep without increasing the time spent in REM sleep or changing arterial pressure.

The distribution of Na^+, K^+-adenosinetriphosphatase in the rat brain is not uniform; relatively high activity of this enzyme is found in the limbic system (Donaldson *et al.*, 1971). An autoradiographic study has revealed that [^{3}H]-ouabain administered intraventricularly is accumulated preferentially in this region; the inhibitory effect of ouabain injected into the lateral cerebral ventricle on the enzyme activity is also observed most significantly in this region. These findings indicate that this region may be a major site for both binding and action of i.c.v. ouabain.

The sensitivity of the limbic system to ouabain is different among sleep states. Baldy-Moulinier *et al.* (1973) have shown that the limbic system of cats becomes most sensitive to ouabain during REM sleep. A low dose of ouabain induces epileptic discharges on the EEG only during REM sleep without arousing the animal; the high dose causes epileptic phenomena during any sleep state and lethal convulsive seizures. In a preliminary study (Tadokoro *et al.*, 1991), we also have confirmed that the chronic i.c.v. infusion of ouabain at a dose of 1 μg kg^{-1} h^{-1} induces epileptic discharges on the EEG only during REM sleep without arousing rats. We therefore selected the smaller doses of 1–100 ng kg^{-1} h^{-1} that do not result in any EEG abnormalities for the present study.

Our previous studies have shown that REM-sleep-related bradyarrhythmias such as sinus arrest, sinoatrial block, and type I (Wenckebach) second-degree atrioventricular block are observed in normal rats (Saito *et al.*, 1983; Otsuka *et al.*, 1986; 1987), and that the frequency of bradyarrhythmias is decreased by vagotomy (Otsuka *et al.*, 1986). Many earlier electrophysiological studies have suggested the importance of the neural activity generated phasically during REM sleep in REM-sleep-related changes in the activity of the autonomic nervous system (Calvo & Fernández-Guardiola, 1984; Kline *et al.*, 1986; Otsuka *et al.*, 1987); the phasic neural activity spreads from the brain stem to the visual cortex and the limbic system. It may be speculated that the phasic neural activity stimulates limbic structures and results in bradyarrhythmias occasionally through drastic vagal activation. Ouabain may prime the genesis of bradyarrhythmias during REM sleep through a permissive action. We therefore speculate that centrally infused ouabain can make limbic

structures prone to be more activated by the phasic neural activity. This proposed mechanism may also explain why 'continuously' infused ouabain increases 'sudden' cardiac pauses only during REM sleep and does not affect average HR. The present study may provide a new view of mechanisms for digitalis-induced arrhythmias during sleep.

In conclusion, centrally administered ouabain aggravated arrhythmias during REM sleep in freely moving rats. We suggest that the CNS activity during REM sleep may be involved in the centrally mediated arrhythmogenic properties of ouabain.

We are grateful to Hiroyoshi Takatsuji of the Medical Research Laboratory for excellent technical assistance.

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(Received January 8, 1993
Revised April 29, 1993
Accepted May 10, 1993)