Epilepsia, **46**(3):367–371, 2005
Blackwell Publishing, Inc.

Changes in Baroreflex Responses of Kindled Rats

Çiğdem Apaydin Kaya, Aydan Ergün Özkaynakçi, M. Zafer Gören, and Filiz Yilmaz Onat

Marmara University Faculty of Medicine, Department of Pharmacology and Clinical Pharmacology, Istanbul, Turkey

Summary: *Purpose:* This study was planned to investigate the baroreflex responses (BRs) in kindled rats during seizure-free period to put forward new data on cardiac autonomic changes in epilepsy.

Methods: Male Wistar rats were randomized into sham-operated (SO) and kindled groups where stimulation and recording electrodes were implanted stereotaxically into the basolateral amygdala and the cortex, respectively. For kindling process, rats were stimulated twice daily at their afterdischarge threshold current and accepted as being kindled after 10 grade 5 seizures. Six to 8 weeks after the establishment of the kindled state, mean arterial pressure (MAP) and heart rate (HR) were evaluated. BR was defined as the ratio of HR response to changes in MAP induced by i.v. nitroprusside (10, 25 μg/kg) or i.v. phenylephrine (10, 25 μg/kg). The sympathetic or parasympathetic component of the BR was evaluated in rats pretreated with atropine or atenolol where phenylephrine or nitroprusside was administered at 25 μg/kg.

Results: Basal MAP and HR values were found to be similar in SO and kindled rats. Phenylephrine increased MAP more in the kindled group ($p < 0.05$), but the HR decreased similarly in both groups. Nitroprusside decreased MAP at similar rates, but the increase in HR was higher in the kindled rats ($p < 0.05$). BRs to phenylephrine and nitroprusside were abolished after pretreatment with atenolol and atropine, whereas phenylephrine- and nitroprusside-induced changes in MAP remained unchanged in both groups.

Conclusions: These results may indicate that amygdaloid kindling affects BRs in long-term seizure-free periods. **Key Words:** SUDEP—Baroreflex responses—Kindling—Epilepsy—Autonomic dysfunction.

The mortality rate is 2 to 3 times higher in the people with epilepsy than in the general population (1). Moreover, increased risk of sudden death occurs in 7.5–17 % of all deaths among patients with epilepsy (2). Although the mechanisms underlying sudden unexplained deaths in epilepsy patients (SUDEPs) have not been clearly established, seizure-related arrhythmias, cardiorespiratory pathology, and autonomic dysfunction have been suggested as the causes of SUDEP (3–6). Several experimental seizure and epileptogenesis models have been used to examine the physiopathologic mechanisms responsible for SUDEP (7–9).

Kindling, an experimental model of temporal lobe epilepsy, is performed by daily application of short trains of high-frequency electrical pulses that induce initially focal but subsequently generalized convulsive seizures (10). It is characterized by the increase of electrographic afterdischarge duration and behavioral complexity produced by the repeated low-density electrical stimulation of several brain areas, mainly the limbic system. In previous studies, immediate effects of kindled seizures on cardiovascular function have shown a seizure-associated increase in blood pressure and bradycardia with a variety of arrhythmias during the seizures in unanesthetized and freely moving rats (10,11). Furthermore, seizure-induced cardiovascular-function changes in kindled animals have been reported to originate from activation of the sympathetic and parasympathetic branches of the autonomic nervous system, suggesting that autonomic dysfunction is a possible mechanism of SUDEP (12). In a recent study, we investigated the short-term effects of kindling epilepsy on γ-aminobutyric acid $(GABA)_A$-receptor antagonist, bicuculline- and *N*-methyl-D-aspartate (NMDA)-induced cardiovascular responses and extracellular levels of noradrenalin, dopamine, GABA, and glutamic acid in the dorsomedial nucleus of the hypothalamus, which plays a vital role in the autonomic activity and physiologic regulatory processes. In addition to decreases in noradrenalin and dopamine levels in the conscious kindled rats, we observed that neither blood-pressure nor heart-rate responses were affected by the acute kindled state (13). The majority of the experimental data demonstrated the immediate acute effects of seizures on blood pressure and heart rate in kindled rats; however, evaluation of the cardiovascular function in a long-term seizure-free period has not been studied and may have potential to explain pathophysiology of SUDEP. Therefore we aimed to evaluate the blood

Accepted September 26, 2004.
Address correspondence and reprint requests to Dr. Filiz Y. Onat at Marmara University Faculty of Medicine, Department of Pharmacology and Clinical Pharmacology, Haydarpasa, 34668, Istanbul, Turkey. E-mail: fonat@marmara.edu.tr

pressure and baroreflex responses (BRs) to i.v. phenylephrine (PHE) and nitroprusside (NP) in chronic and seizure-free kindled rats .

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighing 185–310 g, obtained from the Marmara University Experimental Research and Animal Laboratory, were randomized into sham-operated (SO; n = 7) and kindled groups (n = 8). The rats were housed in a temperature-controlled room (20 ± 3°C) with a 12-h light/dark cycle and allowed free access to food and water. Experimental protocol was approved by the university Animal Care and Use Committee (March 24, 2001).

Electrode placement and kindling

Intraperitoneal ketamine (100 mg/kg) and chlorpromazine (50 mg/kg) were used as anesthetic agents for the stereotaxic surgery performed 7 days before the kindling procedure. The head of the animal was fixed to the stereotaxic apparatus (Stoelting model 51600; Stoelting Co, Wood Dale, IL, U.S.A.), the skull was leveled between lambda and bregma, and the scalp was incised longitudinally where the bregma was used as the reference point. A bipolar stimulation electrode (MS303/2; Plastic One, Roanoke, VA, U.S.A.) was placed into the basolateral amygdala (BLA) in the right hemisphere for kindling. Coordinates were calculated according to the Rat Brain Atlas of Paxinos and Watson (14). Coordinates for the BLA were 2.6 mm posterior and 4.8 mm lateral from the bregma and 8.5 mm vertical from the surface of the skull. For recording the EEG, four stainless-steel screw electrodes were placed bilaterally in the skull over frontal and parietal cortices and connected to a microconnector. The stimulation electrode and microconnector were fixed to skull with dental acrylic cement.

After a recovery period of 7 days, the kindling process was initiated in the kindling group. The afterdischarge threshold (AT) was determined for each rat, and they received electrical stimulation with biphasic square-wave pulses of 80 Hz, each 1-ms duration, for a total period of 2 s. The intensity was increased in 50-μA steps until an AT was elicited. EEG recordings were performed via an EEG transducer (F-51C3; Grass-Telefactor, West Warwick, RI, U.S.A.) connected to an amplifier (P551; Grass-Telefactor) on the polygraph. The right BLA was stimulated twice daily at the current AT until 10 consecutive grade 5 seizures occurred. Seizure grades observed after each stimulus were classified by using Racine's standard five-grade scale: grade 1: facial movements; grade 2: rhythmic head movements, head nodding; grade 3: unilateral forelimb clonus; grade 4: bilateral forelimb clonus and rearing; and grade 5: falling and tonic–clonic convulsion (10). When 10 grade 5 seizures occurred, animals were considered to be kindled. Six to 8 weeks after the achievement of the kindled state, experiments were performed.

In the SO group, a stimulation electrode was implanted 2 mm above the BLA, but no current was applied. Similarly, the experiments were performed 6–8 weeks after the surgery.

Evaluation of cardiovascular parameters

On the day of cardiovascular experiments, arterial and venous catheters (PE-10 tubing connected to PE-50) filled with 1% heparinized saline solution were placed into the iliac artery and vein for measurement of BR and drug administration, respectively, under brief ether anesthesia. The catheters were tunneled through the skin and externalized on the back of the neck. Each rat was placed in a Plexiglas cage, and the experiments were conducted ≥3 h after the surgery, in awake and freely moving animals.

The extension tubing of the iliac artery catheter was connected to a pressure transducer (model P23ID; Grass-Telefactor) for monitoring blood pressure on a polygraph (model 7; Grass-Telefactor). Heart rate was determined by using a tachometer (model 7 P44; Grass Telefactored by arterial pulse pressure.

After a stabilization period, basal arterial blood pressure and heart rate were recorded for ≥30 min. The response of BRs was evaluated by measuring the reflex bradycardia in response to transient hypertension evoked by an i.v. bolus administration of PHE (10 and 25 μg/kg) and by measuring the reflex tachycardia in response to transient hypotension evoked by i.v. bolus administration of sodium NP (10 and 25 μg/kg). After the blood pressure and heart rate returned to baseline values, other drug or dose was administered. PHE doses followed NP doses alternatively.

After determining the BRs, two autonomic blockers were administered to evaluate the involvement of cardiac vagal and sympathetic influences in mediating the baroreflex control of the heart rate. For determining the parasympathetic component of the BRs, the muscarinic receptor antagonist, atropine methyl nitrate (0.5 mg/kg), which does not cross blood–brain barrier, was used to block parasympathetic effects on the heart. Subsequently, a cardioselective β_1 antagonist, atenolol (0.5 mg/kg), was given to determine the sympathetic influence on the heart. Ten minutes after the administration of atropine methyl nitrate and atenolol, PHE or NP was injected at 25-μg/kg doses.

All drugs were dissolved in physiologic saline and given as an i.v bolus injection (0.5 ml/kg) for 10 s.

Histologic verification of the placement of electrodes

After all experiments, rats were decapitated to determine electrode placement; brains were removed and placed in a formalin/sucrose mixture. The brains were sectioned into 50-μm slices with a cryostat and stained with thionine. Only correct electrode placements were

TABLE 1. *Baseline MAP and HR in the SO and kindled rats*

	SO	Kindled
MAP (mm Hg)	108 ± 1.41	108 ± 2.61
HR (beats/min)	370 ± 12.86	350 ± 5.35

MAP, Mean arterial pressure; HR, heart rate; SO, sham operated.

included in the data analysis. The experiments performed with improper electrode placement were not included in the study because of limited number of various regions other than BLA.

Data analysis

All values were expressed as mean ± SEM. MAP was calculated as "1/3 pulse pressure + diastolic pressure." Student's *t* test was used demonstrate the differences between SO and kindled rats for arterial-pressure and heart-rate responses to drugs; $p < 0.05$ was considered being significant. Kruskall–Wallis statistics followed by Dunn's test were used to compare more than two groups.

RESULTS

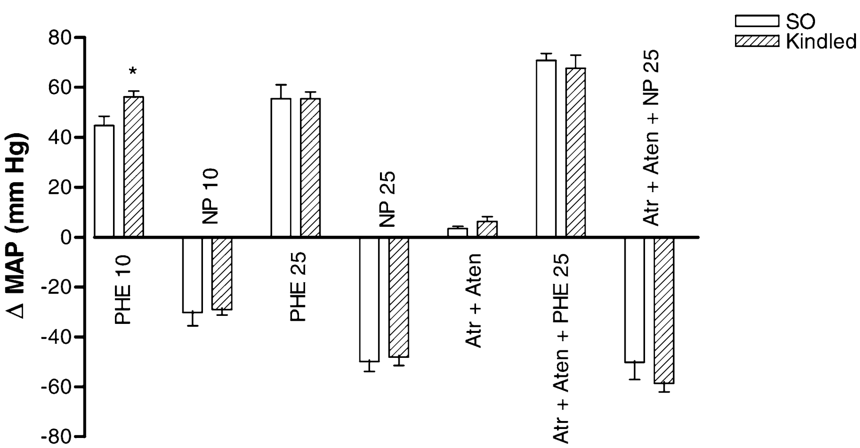
Cardiovascular responses to i.v. administration of PHE or NP in SO and kindled rats

No statistically significant difference was detected in the baseline MAP and heart-rate values of SO and kindled rats (Table 1). Administration of 10 μg/kg of PHE produced an increase in MAP (Fig. 1) and a decrease in the heart rate of SO and kindled rats (Fig. 2). The increase in MAP of kindled rats was shown to be statistically higher than that observed in SO rats ($t = 2.658$; $df = 13$; $p < 0.05$; Fig. 1) but the decrease in heart rate was similar in both groups (Fig. 2). PHE trial at 25 μg/kg produced a similar effect in both SO and kindled groups (Figs. 1 and 2). Collectively, when we applied Kruskal–Wallis statistics followed by Dunn's test to our data, the MAP values of PHE, 10 mg/kg (SO: KW = 13.72; $df = 2$; $p < 0.05$; Kindled: KW = 15.50; $df = 2$; $p < 0.01$) and PHE, 25 mg/kg (SO: KW = 13.72; $df = 2$; $p < 0.01$; Kindled: KW = 15.50; $df = 2$; $p < 0.01$), groups were found to be significantly different from the basal MAP values, but no difference was found between PHE, 10 mg/kg, and PHE, 25 mg/kg groups of sham-operated and kindled rats. Likely, the MAP values after PHE 10 and 25 did not produce a statistically significant effect.

Injection of 10-μg/kg and 25-μg/kg doses of NP produced a decrease in MAP and an increase in heart rate in SO and kindled rats, as shown in Fig. 1 and 2, respectively. Although decrease in MAP was similar with two doses of NP in both groups, heart-rate responses to 10-μg/kg and 25-μg/kg doses were significantly higher in the kindled group than in SO animals ($t = 2.546$; $df = 13$; $p < 0.05$; and $t = 2.268$; $df = 10$; $p < 0.05$, respectively).

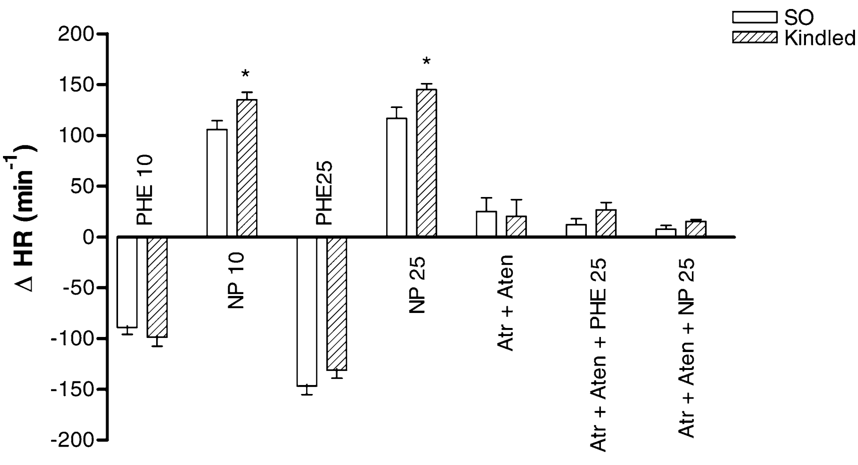
Baroreflex responses in SO and kindled rats pretreated with atropine and atenolol

After i.v. administration of the muscarinic receptor blocker, atropine (0.5 mg/kg) and cardioselective β_1-receptor antagonist, atenolol (0.5 mg/kg), the blood pressure and heart rate remained unchanged for both SO and kindled groups. PHE administered at the dose of 25 μg/kg 10 min after pretreatment with atropine and atenolol produced significant increases in MAP in SO and kindled rats (SO: $t = 20.83$; df = 10; $p < 0.05$; Kindled: $t = 10.24$; df = 14; $p < 0.05$), whereas no decrease in the heart rate was detected in the groups (Figs. 1–3). Subsequently, after stabilization of MAP and heart rate, administration



PHE 10		NP 10		PHE 25		NP 25		Atr+Aten		Atr+Aten + PHE 25		Atr + Aten + NP 25	
108 ± 1.41	108 ± 2.56	109 ± 2.73	108 ± 3.13	107 ± 2.89	110 ± 3.25	108 ± 1.27	109 ± 4.11	109 ± 1.89	106 ± 3.36	110 ± 1.92	111 ± 3.42	103 ± 3.40	109 ± 4.63

FIG. 1. The maximal changes in mean arterial blood pressure (MAP) recordings of sham-operated (SO) and kindled rats in response to intravenous phenylephrine (PHE) or nitroprusside (NP) at 10 and 25 μg/kg. Injections of PHE and NP were repeated at the 25-μg/kg dose after intravenous atropine (0.5 mg/kg) and atenolol (0.5 mg/kg) pretreatment. **Inset:** Mean preinjection MAP ± SEM values (mm Hg) in the order of SO and kindled rats in the corresponding treatment columns.



PHE 10		NP 10		PHE 25		NP 25		Atr+Aten		Atr+Aten + PHE 25		Atr + Aten + NP 25	
370 ± 12.86	350 ± 5.35	371 ± 11.48	345 ± 8.24	394 ± 5.83	360 ± 6.55	373 ± 12.29	359 ± 7.35	381 ± 13.99	370 ± 7.24	403 ± 14.36	400± 13,05	403 ± 12.21	372 ± 23.11

FIG. 2. The maximal changes in pulsatile heart-rate (HR) recordings of sham-operated (SO) and kindled rats in response to intravenous phenylephrine (PHE) or nitroprusside (NP) at 10 and 25 μg/kg. Injections of PHE and NP were repeated at 25-μg/kg doses after intravenous atropine (0.5 mg/kg) and atenolol (0.5 mg/kg) pretreatment. Inset: Mean preinjection HR ± SEM values (min = 1) in the order of SO and kindled rats in the corresponding treatment columns.

of NP (25 μg/kg) produced statistically significant decreases in MAP in both groups, (SO: t = 6.474; df = 8; $p < 0.05$; Kindled: t = 7.932; df = 8; $p < 0.05$), but NP-induced tachycardia was abolished in SO and kindled rats (Figs. 1–3).

DISCUSSION

The results of the present study demonstrate the increased cardiovascular responses to NP and PHE in the seizure-free period of kindled rats when compared with the SO group, suggesting a hyperresponsiveness of cardiac

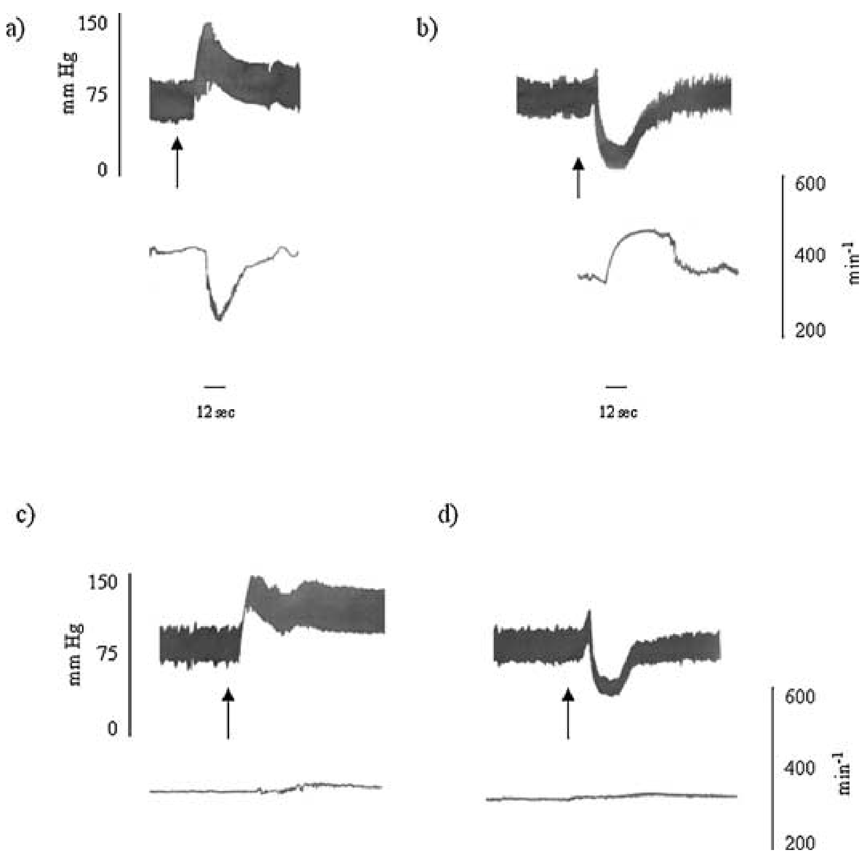


FIG. 3. The traces of blood pressure (mm Hg) and heart rate (beat/min) recordings through a pressure transducer and tachograph connected to a polygraph in response to intravenous phenylephrine and nitroprusside (at 25 μg/kg) before **(a, b)** and after **(c, d)** atropine and atenolol pretreatment (0.5 mg/kg), respectively. The traces were recorded at a chart speed of 25 mm/min.

sympathetic activation and an impaired BR function in the chronic phase of the kindling model of epilepsy. This outcome is consistent with the previous studies indicating that epilepsy affects the autonomic control of cardiovascular homeostasis (15,16). It has been shown that the autonomic function in patients with epilepsy is altered by recurrent interictal and ictal discharges in the limbic structures, which are richly interconnected with areas regulating blood pressure and heart rate. Moreover, the studies testing central cardiovascular regulation in different species demonstrated changes in the interictal autonomic nervous system function. In a previous report, Lathers et al. (17) showed that interictal epileptogenic discharges enhance cardiovascular sympathetic tone more than parasympathetic outflow in pentylenetetrazole-treated cats (17). Likewise, the finding of the greater tachycardia in kindled rats than that observed in SO rats with two doses of NP in the present study is in agreement with Lather's results. Additionally, the findings that PHE- and NP-induced BRs in kindled animals were completely eliminated by the muscarinic receptor blocker, atropine, and β_1-receptor blocker, atenolol, whereas they had no effect on blood pressure responses, show that PHE-induced bradycardia and NP-induced tachycardia are due to central activation of the parasympathetic and sympathetic branches of the autonomic nervous system. BR, regarded as a measure of cardiac vagal reflex activity, quantifies the capability of vagal heart rate activity in response to an increase or decrease in arterial blood pressure (18). BRs receive growing interest because of the prognostic potential as a marker of autonomic activity, since compelling evidence has been provided for the existence of a tight relation between the autonomic nervous system and sudden death (19).

In addition to the acute effects of seizures on the cardiovascular system examined in several experimental models such as kindling, electroconvulsive shock, and topical application of penicillin-G (10,20,21), the finding of the present study that the rats had neither spontaneous nor evoked kindled seizures before or during the experiments implies that cardiovascular changes are not associated with seizure activity. Therefore our study gives a complementary approach to evaluate the hypothesis of cardiovascular and autonomic dysfunction in the risk of SUDEP in the chronic period. However, some reports pointed out that the changes in blood pressure, heart rate, and cardiac rhythm occur independent of the motor activity seen during the seizures, but epileptic seizures are known to be accompanied by profound autonomic dysfunction, such as cardiovascular function and respiratory, metabolic, and gastrointestinal motility changes during the seizure state.

In conclusion, our present results demonstrate that the increased responses to PHE and NP in kindled animals support the hypothesis of baroreflex dysfunction during the chronic seizure-free period, but further studies using other models of epilepsy also are required to determine the role of kindling itself or epilepsy in altered BRs.

Acknowledgment: This study supported by a grant from Marmara University Scientific Research and Projects Commission (Project number: SAĞ-078/051201).

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