European Review for Medical and Pharmacological Sciences 2014; 18: 1454-1457

Urethane suppresses renal sympathetic nerve activity in Wistar rats

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Abstract. – AIM: To investigate the effects of urethane on renal sympathetic nerve activity (RSNA) in Wistar rats.

MATERIALS AND METHODS: 46 rats were randomly allocated in two groups: group A in which rats were injected with urethane; group B in which barbital sodium was used as a control. The changes of RSNA, blood pressure (BP) and heart rate (HR) of each group were evaluated and analyzed.

RESULTS: Compared to the control group, the value of RSNA, BP and HR were all significant decreased in rats of group A after urethane injection ($p < 0.05$).

CONCLUSIONS: These results suggest that urethane could affect RSNA through somatosensory system.

Key words:
Urethane, Anesthetics, Renal sympathetic nerve activity, Blood pressure, Heart rate.

Introduction

As an anesthetic administration method, urethane is widely used in animal experiments. It has variant effects on physiological function. Generally, carbamates, including urethane, bind to the active site of acetylcholinesterase in a similar manner to acetylcholine, which promotes the rupture of the ester bond and the formation of carbamyl-acetylcholinesterase[1,2]. This reaction inhibits the activity of acetylcholinesterase, leading to the accumulation of acetylcholine and failure of transmission of nerve impulses in experimental animals[3-5]. In this study, we examined the effect of urethane on renal sympathetic nerve activity (RSNA) of Wistar rats by using power spectrum analysis. Our data indicate that urethane may suppress RSNA in anaesthetized rats.

Materials and Methods

Animal surgery

A total of 46 Wistar rats (280-330 g) were randomly divided into two equal groups. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the First Affiliated Hospital of Xinxiang Medical University. These rats were fasted the day before experiments. Urethane was used as the anesthetic. For group A (urethane), rats were anaesthetized with enflurane, followed by the urethane administration with a micro-infusion pump via femoral vein. Both blood pressure and heart rate were monitored during the procedure. Meanwhile, corneal reflex and hind pain responses were checked every 10 min to keep the anaesthetization working. According to the experiment require, urethane was steady injected until the plasma concentration reached 600 mg/kg. During the operation, rectal temperature was maintained at 37°C. Left kidney was exposed in order to get an idea signal, and then isolate renal nerve, fixed the distal terminal on a bipolar electrode which covered with warm paraffin oil. For each sample, the nerve activity was recorded at 1 kHz frequency for 164s.

For group B (conscious), the rats were first anaesthetized by intraperitoneal injection of barbital sodium (60 mg/kg), and additional barbital sodium was given upon to respiratory rate and corneal reaction. Renal nerve was isolated and fixed on a bipolar recording electrode, followed by seal with silica gel. Animals were injected with analgesic buprenorphine hydrochloride (30 μg/kg) and returned back to cages. Reaction responses were checked at least twice a day. The conduit and electrode were placed on a mechani-

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cal instrument which contains a rotation system to let the animals move comfortably in cages. 3 days later, the recording was performed by using the same setting as in rats from group A. The filtered and unfiltered signal of RSNA, and the signal of blood pressure were all recorded by the instrument.

Experimental procedures and data collection

Briefly, baseline of blood pressure and RSNA were firstly recorded for 3.5 min. Then, blood pressure and the signal of RSNA were recorded after electrical stimulation of the anterior limb with 0.5 Hz, 0.8 Hz, 1 Hz, and 1.6 Hz current (15 V, 0.2 ms). Electrical stimuli were generated by an electronic oscillator with sine wave. After the first round recording of the baseline and wait until to all the physiological parameters became stable (usually within 15-30 s), next 3.5 min recording would be started. The signal of RSNA was recorded after filtration (high pass: 100 Hz, low pass, 1000 Hz) and amplification ($10^5\times$). The reading of blood pressure and RSNA were displayed on a two-channel oscilloscope. During the recording, LabVIEW acquisition systems were set to integrate the signal of RSNA once a second, and then calculated the average values of blood pressure and nerve activity during the recording time. At the last minute of recording, the value of blood pressure and the signal of nerve activity were recorded by high-pass filter (1000 Hz). The signal of RSNA was rectified to eliminate the DC noise. The data at the last minute were divided into ten segments; each of them contains about 6000 data points. Then, the average sequence was calculated, generating 6000 data points. The obtained average sequence was divided into three segment, each containing 3000 data points and corresponding to 305 sampling periods. Two parameters were obtained from power spectrum: gross power and the power percentage of cardiac rhythm frequency. First, the area between 0-10 Hz below the curve was calculated to get gross power. According to it, the heart rate was determined by the power spectrum of blood pressure. The area which represented the heart rate between ±0.1 Hz was defined as the absolute value of the power component of RSNA on heart rate. This absolute value was divided by gross power to get the power percentage of cardiac rhythm frequency, which reflected the activities of pressoreceptor circuits.

Statistical Analysis

The average pressure whose unit of measurement was mmHg represents the variable of blood pressure. The unit of measurement of RSNA was mVS-1, and its present form was Mean±SEM. ANOVA analysis was used to compare the difference between the two groups. The data of recording value in electrical stimulation experiment was compared with the value recorded before the stimulation. $p < 0.05$ was defined as statistical significant difference.

Results

Dose-dependent effects of urethane on blood pressure, heart rate, and RSNA in group A

Urethane injection caused decrease of blood pressure, heart rate, and RSNA in a dose-dependent manner. When the plasma concentration of urethane reached 100 mg/kg, 200 mg/kg, 400 mg/kg, and 600 mg/kg, RSNA decreased to 7%, 14%, 17%, and 27 %, respectively ($p < 0.05$). Furthermore, the blood pressure and heart rate were also suppressed due to the administration of urethane ($p < 0.05$) (Table I).

Effects of electrical stimulation on RSNA in rats from group A

When plasma concentration of urethane reached 200 μg/kg, 400 μg/kg, and 600 μg/kg, we stimulated the forelimb nerve with 0.5 Hz, 0.8 Hz, 1.0 Hz, and 1.6 Hz current, and then recorded the total energy of renal nerve. As shown in Table II, urethane treatment could significantly decrease the total energy of RSNA. Interestingly, the inhibitory effect of urethane was elevated upon to the increase of plasma concentration. The most significant increase (more than 50%) appeared at 1.6 Hz electrical stimulation ($p < 0.01$).

Table I. The change of HR, BP and RSNA by Urethane with different concentrations

Value/ conc.	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg
BP	-3%	-9%	-16%	-34%
HR	-6%	-10%	-19%	-31%
RSNA	-7%	-14%	-17%	-27%

Table II. The change of RSNA in group A by current stimulation with different frequency.

Conc./f	0.5 Hz	0.8 Hz	1.0 Hz	1.6 Hz
200 μg/kg	-3%	-5%	-8%	+14%
400 μg/kg	-7%	-11%	-17%	+28%
600 μg/kg	-12%	-19%	-34%	+57%

Effects of current stimulation on RSNA in rats from group B

The same current stimulation was performed in rats from group B. The results showed that in compared to the group A, both blood pressure and heart rate were upregulated in group B ($p < 0.05$). Stimulation at 0.5 Hz, 0.8 Hz, and 1.0 Hz could suppress RSNA; however, 1.6 Hz stimulation can increase it ($p < 0.05$).

Comparison of blood pressure and heart rate between rats from group A and group B

When the plasma concentration of urethane was 400 μg/kg, 0.5 Hz and 1.6 Hz current stimulation decreased the HR by 49% and 57% in group A rats. This is similar to the effect on group B (decreased by 46% and 51%) ($p > 0.05$). When the plasma concentration of urethane was 300 g/kg, the same current stimulation caused a more significant decrease of HR in rats from B compared to rats from A. When the plasma concentration reached 600 μg/kg, current stimulation caused a more significant decrease of HR in rats from A ($p < 0.05$).

Discussion

A combination of urethane and chloralose are always used in animal experiment for anesthetic administration. The pharmacological function of chloralose is similar to morphine. However, previous studies demonstrated that could affect the activity of peripheral sympathetic nerve, total peripheral resistance, blood pressure, and heart rate. Rat and rabbit models also suggested that the administration of urethane in central neural system can lead to inhibitory effect of RSNA[6,7]. In addition, studies in rabbits demonstrated that urethane inhibited the baroreflex of RSNA in a dose-dependent manner[9].

There are two purposes in this study. First, to observe the changes of blood pressure, heart rate, and RSNA in Wistar rats injected with urethane of different concentration. This includes the changes of physiological parameters and the modality of RSNA. In our study, we attempt to exam the effect of urethane on the energy distribution of RSNA, especially that of heart rate. Secondly, to exam whether the activation of renal nerve and the distribution of energy after stimulation of sensory receptors are affected by the action of urethane on the central nervous system.

Sympathetic neural activity could be enhanced by the current stimulation with different frequency. On the other hand, the activity of all the RSNA, blood pressure and heart rate can provide another confirmation of this phenotype which might be caused by the depolarization of nerve fiber of skin and muscle[10-12]. Some previous study demonstrated that the distribution pattern of neural power could be alerted by current stimulation. In this study we found that although the total neural power was increased by current stimulation, the one of heart rate was lower than control. In contrast to heart rate, the power of RSNA was significantly increased. As the pressure feedback regulation of RSNA is more sensitive, the power of RSNA with current stimulation is stronger compared to heart. So this study demonstrated that the effect of RSNA regulation is not only depending to the neural activity, but also the distribution of total power and the sensitiveness of each organ[13-16]. There is barely report about correlation between urethane and parasympathetic neural activity. However, according to the response of sympathetic neural activity in our study, it could be still believed that there exist some feedback connections of parasympathetic activities in other body systems.

Although the basic line of blood pressure and heart rate were depressed by urethane before the current stimulation, the stimulation still can enhance the two values which can reflect the general neural activity. Nonlinear static procedure might provide a better understanding of the relationship between the effect of urethane and RSNA[17,18].

Conclusions

Urethane leads to a complicate spatiotemporal effect in RSNA. This study showed that urethane is an important anesthetic reagent in RSNA.

Conflict of interest

The Authors declare that they have no conflict of interests.

References

1) Pincus SM. Approximate entropy as a measure of system complexity. Proc Natl Acad Sci U S A 1991; 88: 2297-2301.
2) Richman J, Moorman J. Physiological time-series analysis using approximate entropy and sample entropy. Am J Physiol Heart Circ Physiol 2000; 278: H2039-H2049.
3) Tang XJ, Tian X, Yang Z, Zhang T. Complexity measurements of Electroencephalograph (EEG) recordings using sample entropy algorithm in patients with temporal lobe epilepsy. Acta Biophysica Sin 2004; 20: 382-392.
4) Nagabukuro H, Okanishi S, Imai S, Ishichi Y, Ishihara Y, Doi T. Effects of TAK-802, a novel acetylcholinesterase inhibitor, on distension-induced rhythmic bladder contractions in rats and guinea pigs. Eur J Pharmacol 2004; 485: 299-305.
5) Kurz C, Baranowska U, Lupi ski S, Göthert M, Malinowska B, Schlicker E. Urethane, but not pentobarbitone, attenuates presynaptic receptor function in rats: a contribution to the choice of anaesthetic. Br J Pharmacol 2009; 157: 1474-1482.
6) Xu H, Aibiki M, Seki K, Ogura S, Yokono S, Ogli K. Effects of induced hypothermia on renal sympathetic nerve activity and baroreceptor reflex in urethane-anesthetized rabbits. Crit Care Med 2000; 28: 3854-3860.
7) Barman SM, Gebber GL. The posterior vermis of the cerebellum selectively inhibits 10-Hz sympathetic nerve discharge in anesthetized cats. Am J Physiol Regul Integr Comp Physiol 2009; 297: R210-R217.
8) Costa M, Goldberger AL, Peng CK. Multiscale entropy analysis of complex physiologic time series. Phys Rev Lett 2002; 89: 068102.
9) Costa M, Goldberger AL, Peng CK. Multiscale entropy analysis of biological signals. Phys Rev E Stat Nonlin Soft Matter Phys 2005; 71: 021906.
10) Zhang T, Johns EJ. Chaotic characteristics of renal nerve peak interval sequence in normotensive and hypertensive rats. Clin Exp Pharmacol Physiol 1998; 25: 896-903.
11) Li D, Jin YX, Yang Z, Zhang T. Analysis of multifibre renal sympathetic nerve recordings. Lecture Notes in Computer Science 2006; 3973: 734-739.
12) Nakamura A, Imaizumi A, Kohsaka T, Huang C, Huang C, Johns EJ. The actions of Shiga toxin-2 administration into the brain on renal sympathetic nerve activity. Clin Exp Nephrol 2012; 16: 382-388.
13) Zhu S, Ma KK. A new diamond search algorithm for fast block-matching motion estimation. IEEE Trans Image Process 2000; 9: 287-290.
14) Salman IM, Sattar MA, Abdullah NA, Ameer OZ, Basri F, Hussain NM, Yam MF, Swarup KR, Rathore HA, Kazi RN, Hye Khan MA, Johns EJ. Role of renal sympathetic nervous system in the control of renal potassium handling. J Nephrol 2010; 23: 291-296.
15) Davatzikos C, Bryan N. Using a deformable surface model to obtaina shape representation of the cortex. IEEE Trans Med Imag 1996; 15: 785-795.
16) Snel JG, Venema HW, Grimbergen CA. Deformable triangular surfaces using fast 1-D radial Lagrangian dynamics—segmentation of 3-D MR and CT images of the wrist. IEEE Trans Med Imag 2002; 21: 888-903.
17) Feng ZY, Zheng XX. The dynamic change of rat EEG s complextity and power spectrum under different anesthetized depth. Chinese J Biomed Eng 2004; 23: 87-91.
18) Li Y, Qiu J, Yan R, Yang Z, Zhang T. Weakened long-range correlation of renal sympathetic nerve activity in Wistar rats after anaesthesia. Neurosci Lett 2008; 433: 28-32.