

Immediate effects of increased blood pressure on pattern of resting discharge in renal postganglionic neurons

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Abstract: In experiments performed on anaesthetized rabbits the pattern of resting activity in renal postganglionic neurons during sustained increase in blood pressure (BP) was investigated. Resting discharge was recorded in 39 single units. Sustained increases in BP by about 20 mm Hg were evoked by i.v. infusion of phenylephrine (15 µg/kg/min). From interspike-interval histograms accumulated for each neuron, the shortest, preferred and longest interspike-intervals were calculated. The mean discharge rate was also measured. During elevation of BP the shortest interspike-interval lengthened from control value of 7.2 ± 0.2 ms (\pm S.E.) to 17.4 ± 8 ms, preferred interval increased from 28.3 ± 5.7 ms to 80.7 ± 12.9 ms, and longest interval lengthened from 6440 ± 396 to 7431 ± 387 ms. The discharge rate decreased from 1.437 ± 0.2 to 0.973 ± 0.2 spikes/s. All changes were statistically significant ($P < 0.05$). Elevated BP lowered correlation coefficient between the shortest interval and discharge rate from $r = -0.347$ ($P = 0.03$) to $r = -0.186$. Immediate effects of elevated BP on pattern of discharge of single renal neurons most probably resulted from the central effect of phenylephrine on altered interactions between cells generating activity in vasomotor neurons.

Key words: resting discharge, renal sympathetic neurons, increased blood pressure, phenylephrine, pattern of discharge

INTRODUCTION

The resting discharge of sympathetic neurons has been analyzed from the point of view of its modulations related to the pulse pressure and respiration (cardiac and respiratory modulations) or distribution of intervals between its spike potentials [1, 2, 3]. It was recently found that the elevation of blood pressure level enhances cardiac modulation of the resting discharge in renal postganglionic neurons. The observed effect is probably due to a stronger barrage of baroreceptor impulses impinging on medullary neurons which are the site of generation of the resting discharge in sympathetic neurons [4]. To obtain a deeper insight into the mechanisms of tonic baroreceptor input on the sympathetic discharge we studied the effect of phenylephrine-induced increase in blood pressure on the pattern of discharge in renal postganglionic neurons derived from their interspike-interval histograms.

MATERIAL AND METHODS

The experiments were carried out on adult rabbits. The animals were anaesthetized with urethane (0.8 g/kg, i.v.) and alpha-chloralose (0.03 g/kg, i.p.). A steady level of anaesthesia was maintained by additional doses of alpha-chloralose (3-4 ml/h of 1% solution given i.v.). The responses to cutaneous stimuli were monitored to ensure a proper level of anaesthesia.

A cannula was placed in the jugular vein for infusion of drugs and fluids. The trachea was intubated, and the animals breathed spontaneously. Arterial blood pressure and the standard electrocardiogram were monitored continuously. Respiratory activity was recorded as changes in endotracheal pressure. The exposed tissues were covered with a pool of mineral oil. The temperature of the animal and of the oil was kept close to 37° C by means of heating lamps. The left renal nerve was exposed, cut, and its central end de-sheathed. The resting discharge was recorded from bundles of the renal nerve by means of fine platinum wire electrodes. The nerve signals were amplified and band pass filtered (1 Hz – 3 kHz). From this discharge the activity of single neuron was obtained. It was monitored on an oscilloscope and used to assess the stability of background discharge. This activity was observed during 30 min. If no significant changes in the discharge rate of single neurons were observed, the experiment proper began. Resting activity was recorded in control conditions, and then during intravenous infusion of phenylephrine at a dose of 15 µg/kg/min that produced the sustained increase in arterial BP of about 19-25 mm Hg, as compared with the control value. The duration of recordings in both instances was 10 min. All signals were digitized with 12-bit accuracy and sampled at various frequencies using a 1401plus interface and Spike2 software from CED. The neural signals were sampled at 10 or 20 kHz. The sampling rate for arterial pressure, ECG and endotracheal pressure signals was 250 Hz. Spike sorting was performed on-line using template matching algorithm. All signals were stored on a computer. Spike potentials of single neurons were converted into standard pulses. They were used off-line to construct interspike-interval histograms. The length of the window of histograms was 10 sec and the bin width 2 ms. Mean rate of discharge was expressed as spikes/s.

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Received: 26 March 2008; accepted: 25 June 2008

RESULTS

The resting activity of 39 single sympathetic neurons was recorded. The pattern of their resting discharge was described by 4 parameters calculated from interspike-interval histograms. These were: a) the shortest likely interspike-interval, b) the preferred interspike-interval that corresponded to the highest peak of the histogram, c) the longest interspike-interval and d) the spread of a histogram that was the time between the shortest and the longest interspike-interval. A fifth studied parameter, e) was the mean discharge rate measured at the time of compiling the interval histograms.

The response of the resting discharge in a single renal sympathetic neuron to maintained increase in arterial BP is shown in Figure 1. In control conditions the shortest interspike-interval was 5 ms, preferred interspike-interval amounted to 10 ms and the longest interval was 6,350 ms (Figure 1A). During steady elevation of BP, the shortest interspike-interval was lengthened to 20 ms and the preferred interval increased to 40 ms. The longest interspike-interval amounted to 7,240 ms (Figure 1B). The discharge frequency of the studied renal neuron decreased from 2.1 spikes/s to 0.9 spikes/s.

In control conditions the mean value of the shortest interspike-interval was 7.2 ± 0.2 ms ($x \pm S.E.$) (range from 4-11 ms). The mean preferred interspike-interval amounted to 28.3 ± 5.7 ms (range from 6-184 ms), and the longest interval was 6440 ± 396 ms (range from 1,398-9,799 ms). The spread of interspike-interval histogram was 6423 ± 398 ms and ranged from 1,392-9,792 ms. The mean rate of discharge amounted to 1.44 ± 0.2 spikes/s (range from 0.3-6.1 spikes/s). Analysis of the relationships between the studied parameters showed that 5 out of 10 correlation coefficients were statistically significant. These were: the relationships between the shortest and preferred intervals ($r = 0.356$; $P = 0.026$), between the shortest interval and the discharge rate ($r = 0.347$; $P = 0.03$), and between the longest interval and the discharge rate ($r = -0.841$; $P < 0.001$). Also significant were the relationships between the spread of a histogram and the longest interval

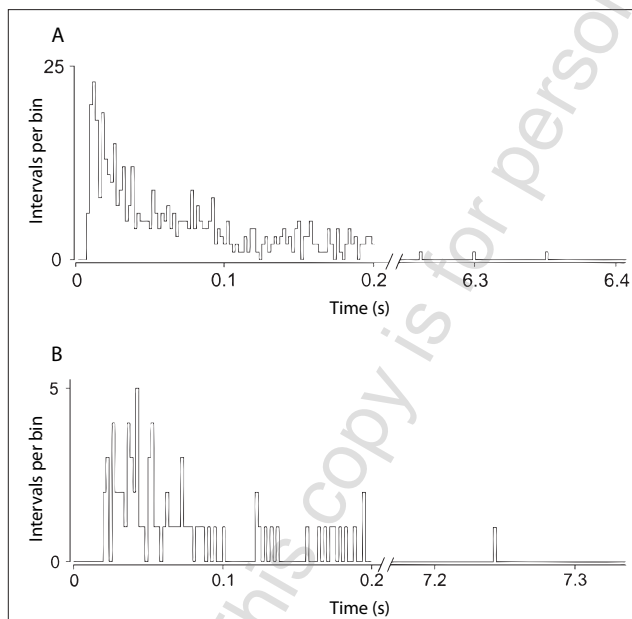


Figure 1 Interspike-interval histograms of ongoing discharge in single renal sympathetic neuron in control conditions (A), and during maintained elevation of arterial blood pressure (B). Note substantial lengthening of the shortest and preferred interspike-intervals in B.

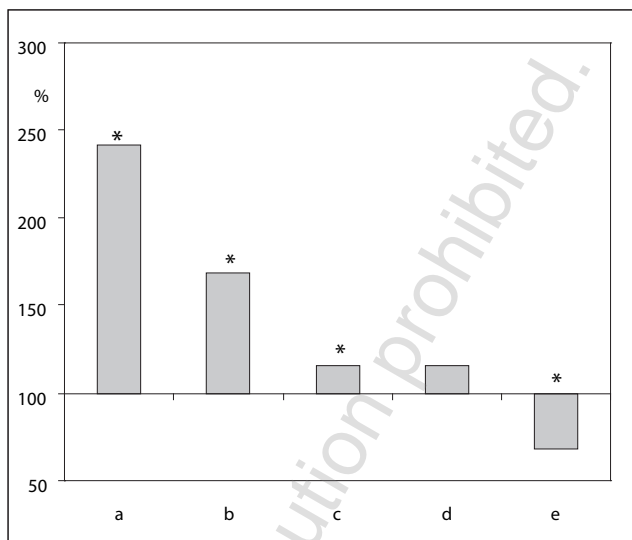


Figure 2 Effect of phenylephrine-induced sustained increase in arterial blood pressure on direction and size of 5 parameters of the resting discharge in renal neurons. The size of parameters in control conditions was taken as 100.

a – shortest interspike-interval
b – preferred interval
c – longest interval between spikes
d – spread of a histogram
e – mean rate of resting activity
* $P < 0.05$

($r = 0.98$; $P < 0.005$), and between the spread of histogram and the discharge rate (-0.842 ; $P < 0.005$).

Sustained increase in BP induced by infusion of phenylephrine evoked important changes in the size of the studied parameters. The shortest interspike-interval increased to 17.4 ± 8 ms (range from 5-320 ms), the preferred interval lengthened to 47.7 ± 12.9 ms (range from 7-435 ms.) and the longest interspike-interval increased to 7431 ± 387 ms (range from 1,818 – 9,951 ms). All these changes were statistically significant ($P < 0.05$). The lengthening of the spread of a histogram to 7401 ± 383 (range from 1,812 – 9,915 ms) was statistically non-significant. On the other hand, the decrease in the discharge rate to 0.973 ± 0.2 spikes/s (range from 0.3-6.1 spikes/s) was statistically significant ($P = 0.022$).

The changes in the size and direction of the studied parameters of the resting discharge occurring during steady elevation of BP are shown in Figure 2. The means in control conditions are taken as 100. Phenylephrine has much larger effects on the shortest and preferred interspike-intervals (parameters a and b) while the changes of other parameters are much smaller.

The number of the significant correlations between the analyzed parameters of the resting activity during elevation of BP decreased from 5 to 4. Statistically significant remained the relationships between: the shortest and preferred interspike-intervals ($r = 0.549$), the longest interval and the discharge rate ($r = -0.892$), and also between the spread of a histogram and the longest interval ($r = 0.999$) and the spread of a histogram and the discharge rate ($r = -0.891$). The relationship between the shortest interval and the discharge rate became statistically non-significant ($r = 0.257$; $P > 0.05$).

DISCUSSION

The results of our experiments indicate that the elevation of BP significantly increased 4 out of 5 parameters derived

from the interspike-interval histograms. The lengthening of the analyzed parameters was not uniform because shorter interspike-intervals were subjected to larger alterations than the longer ones. These findings suggest differentiated effect of phenylephrine-induced increase in BP on parameters making up the pattern of ongoing discharge. The simplest explanation of these findings is that the rise of BP enhances tonic activity of aortic baroreceptors which in turn affect the activity of the vasomotor centre.

In our previous studies on the resting discharge in sympathetic neurons we attempted to establish the effect of exclusion of these receptors by section of the aortic nerves [5]. This procedure eliminated the tonic effect of the aortic baroreceptor input on the vasomotor centre and thus could have the opposite effects on the analyzed interspike-intervals. However, following section of the aortic nerves, the shortest and the longest interspike-intervals were shortened, but the preferred interval was increased. Since these experiments were carried out on vagotomized animals, they cannot be directly compared with the present studies. On the other hand, manipulating the baroreceptor input had a much more unambiguous effect on the correlations between the analyzed parameters. It was found that the elevation of BP decreased the number of significant correlations from 5 to 4, while severing of the aortic nerves increased their number from 3 to 9. These data indicate the importance of the tonic baroreceptor input in determining the strength of the relationship between parameters derived from the resting discharge.

Imaizumi et al. [6] compared the effects of 1-3 min infusion of phenylephrine on BP and multiunit recordings of the renal nerve activity. After discontinuation of the infusion, the elevated BP returned to control level but inhibition of the renal discharge persisted for 1-5 min. These findings and the results of experiments with isolated carotid sinus led the authors to conclude that phenylephrine has a central sensitizing effect on baroreflex control of renal nerve activity. The medullary vasomotor centre (and specifically RVLM) seems to be the most probable site of this action. RVLM generates the resting discharge in the renal neurons and it is conceivable that the phenylephrine-induced augmentation of excitability of its neurons can differentially affect the interspike-intervals between action potentials making up the pattern of discharge of single renal units.

It was recently found that the sustained increase in BP produced by phenylephrine altered the functional connectivity between neurons producing the resting discharge in the renal

neurons [7, 8]. The cause of these changes is not yet clear but it is plausible that they are related with the central effect of phenylephrine.

CONCLUSIONS

The phenylephrine-induced elevation of arterial blood pressure evoked differentiated effects on parameters derived from the interspike-interval histograms of the resting discharge of the renal neurons, more increasing the shortest and preferred interspike-intervals than the longest intervals. It also decreased the number of statistically significant correlations between studied parameters. It may be suggested that these changes are produced by the central effect of phenylephrine which is probably exerted at the level of the vasomotor centre.

REFERENCES

1. Dorward PK, Burke SL, Jänig W, Cassell J: Reflex responses to baroreceptor, chemoreceptor and nociceptor inputs in single renal sympathetic neurons in rabbit and the effects of anaesthesia on them. *J Auton Nerv Syst* 1987, **18**, 39-54.
2. Jänig W: Pre- and postganglionic vasoconstrictor neurons: differentiation, types and discharge properties. *Ann Rev Physiol* 1988, **50**, 525-539.
3. Mannard A, Polosa C: Analysis of background firing of single sympathetic preganglionic neurons of cat cervical nerve. *J Neurophysiol* 1973, **36**, 398-408.
4. Tarnecki R, Lupa K, Pencuła M, Jakubiak M, Warchulińska A, Niechaj A: Cardiac and respiratory modulation of ongoing sympathetic activity during sustained increase in arterial pressure. In: *Molecular and Physiological Aspects of Regulatory Processes of the Organism. Materials of 15th Internat Symp Pol Netw Mol Cell Biol UNESCO/PAS.* (Ed: Henryk Lach. Pedagog Univ, Cracow 2006, 531-532.
5. Lupa K, Wójcik G, Kruk A, Tarnecki R, Niechaj A: Pattern of ongoing discharge of single renal sympathetic neurons in the rabbit. *Arch Physiol Biochem* 1997, **105**, 456-466.
6. Imaizumi T, Brunk SD, Gupta BN, Thames MD: Central effect of intravenous phenylephrine on baroreflex control of renal nerves. *Hypertension* 1984, **6**, 906-914.
7. Warchulińska A, Lupa K, Pencuła M, Niechaj A: The effect of phenylephrine on interactions between sympathetic postganglionic neurons. In: *Molecular and Physiological Aspects of Regulatory Processes of the Organism. Materials of 14th Internat Symp Pol Netw Mol Cell Biol UNESCO/PAS.* (Ed: Henryk Lach Pedagog Univ, Cracow 2005, 517-518.
8. Lupa K, Tarnecki R, Pencuła M, Niechaj A: Functional connectivity between neurons generating resting discharge in renal sympathetic neurons in the rabbit. *Auton Neurosci Basic and Clin* 2002, **101**, 23-31.