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MODULATION OF SPONTANEOUS ACTIVITY OF RAT URETER WITH HISTAMINE

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ABSTRACT

The nature of histamine influence on the slow wave and spike spontaneous activity of both kidney and ureteral peribladder zone with bordering area of bladder was investigated. The regulatory role of histamine was expressed in activation of primary pacemaker region rhythmogenesis localized around pyeloureteral anastomosis; the duration of action potentials genesis might also increase. Histamine also contributed to improvement in conductivity of the propagating wave of excitation to the bladder. The frequency of slow wave activity *of* the ureteral distal *part* slowed under these conditions*: the oscillations we*re stretched, became irregular *and were* completely inhibited further on.

At the same time, rhythm disturbance of spike activity coordinated with the vibrations *expressed in* correlation of each action potential only with certain phases of slow waves was revealed. Apparently, *the segment of* the ureter peribladder zone became a conductor of electrical activity for propagating waves and thus *was* coordinated with the main perirenal rhythmogenesis.

Morphological analysis of histamine effects revealed intense staining of cellular elements of the muscular layer in the regions of ureter and bladder renal section, indicating a high functional status of these areas. Indeed, as shown in the present study, histamine facilitates the spread of spikes to the bladder.

KEYWORDS: . ureter, pyeloureteral anastamosis, peribladder zone, spontaneous activity, automatism, histamine, slow waves.

INTRODUCTION

Electrophysiological studies on pyeloureteral pelvis of ureter in cats, rats and guinea-pigs revealed the presence of slow oscillations of membrane potential [*Bakunts S., 1972; Kazaryan K. et al., 1995; Mc Hale N. et al., 2006*] that provide the subsequent origin of action potentials and their corresponding peristaltic waves down to the bladder. Further topographical study of the organ revealed the presence of a whole spectrum of rhythmogenic autonomous zones different from perirenal area, the most active of which is peribladder zone [*Kazaryan K. et al., 2001; Kazaryan K. et al., 2005*].

If myogenic rhythm plays a major role in causing motility of smooth muscles (peristalsis), the nervous and humoral control are necessary for modulation and coordination patterns of contractility within both certain local areas, and the whole organ [*Bortoff A., 1976; Santicioli P. et al., 1998*]. Among the variety of physiologically active compounds changing the excitability of the membrane of ureteral smooth muscle tissue, histamine is of certain interest due to its ability not only to regulate spontaneous activity, but create a slow pacemaker oscillations of membrane potential as well [*Shuba M., 1977; Bennedito S. et al., 1991*]. Moreover, in studies conducted in guinea-pig ureter the role of histamine in activation of latent pacemakers of the initially silent middle zone of the organ and origination of resulting spike activity was also revealed [*Kazaryan K. et al., 2001; Kazaryan K. et al., 2005*]. Such regulation is associated on the one hand with a high prevalence of H_1-histamine receptors in the tissues of this organ, and on the other hand with the presence of mast cells capable of releasing histamine [*Ugaily-Thuoesius L. et al., 1988; Dodel R. et al., 1996*].

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The effect of histamine on spontaneous activity of renal pelvis of guinea-pigs has been revealed by electrophysiological and morphological studies [*Kazaryan K. et al., 2003*].

Based on the definite relationship between the slow-rhythmogenesis of ureteral distal area and the electrical activity of lower zones [*Kazaryan K. et al., 2005*] obtaining the morphological pattern of pacemaker cells localization of in the bladder and the area bordering to ureter generates undoubted interest. In nowadays literature such studies are lacking.

The purpose of this research was to study the spontaneous activity of both ureteral peribladder zone, and the bordering area of bladder, as well as their relationship with automaticity of pyeloureteral anastomosis under the influence of histamine.

MATERIAL AND METHODS

All experiments were *in situ* carried out in male rats (body weight: 300-350 *g*) intraperitoneally anesthetized by Nembutal (50-55 *mg/kg*). The ureter was denervated by section of the splanchnic and pelvic nerves roots. The slow-wave biopotentials of the ureteral perirenal area were allotted with silver monopolar ball electrodes inserted into the area of the pyeloureteral anastomosis via the kidney parenchyma. The spreading spike bursts were registered with bipolar electrodes. The slow-wave activity of the peribladder zone was recorded by inserting the silver ball electrode into the area of anastomosis of ureter with urinary bladder and spike activity of bladder by bipolar electrode from the surface of the organ. The bioelectrical activity was recorded on 8-channel electroencephalograph (EEG-8 S, Hungary). The presented recording of individual experiments represents data obtained in 8–9 animals. Histamine (Sigma–Aldrich, Germany) was used. The initial solution was prepared in distilled water. The subsequent dilutions were performed in isotonic sodium chloride. The preparation was injected into the femoral vein (by 0.2 *mL* as water solution) in different concentrations: 10^{-6}, 10^{-5}, and 10^{-4} *Mol/L*. One concentration was used in each experiment.

Morphohistochemical studies were performed by the orthophosphates detection method [*Meliksetyan I. et al., 1990*]. In this study the histochemical method based on identification of phosphorus-containing compounds, which are known to occupy key positions in the energy exchange processes of cells, was used. This allows tracking the smallest functional changes in cellular structures of the organ.

All procedures were performed according to "principles of laboratory animal care" (NIH publication № 85-23 revised 1985), as well as the specific rules provided by the Yerevan State Medical University on animal care and use. Every effort was made to minimize animal usage and discomfort.

The analysis of results was carried out using modern packages LabView software and Origin-8.

RESULTS

According to the electrophysiological analysis of the upper urinary tract, strongly rhythmic excitatory waves of ureteral smooth muscle tissue originate in pyeloureteral area in the form of slow-wave oscillations of membrane potential, which, subsequently, in the distal direction along the ureter (within 2-3 *mm*) become the spike activity, extending to the bladder [*Bakunts S., 1970; Meini S. et al., 1995; Kazaryan K. et al., 2005*]. Figure 2A shows a typical pattern of activity in the form of slow-wave oscillations and the spikes emerging on their base in norm. The subsequent injection of histamine into the femoral vein of the animal at a concentration of 10^{-4} *Mol/L* leads to a sharp change of certain characteristics of both types of electrogenesis. The increasing rhythm of both slow waves and action potentials is observed: 24.5±2.6 *oscill/min* (n=9) increase to 44.3±4.2 *oscill/min* (n=12) (Figure 1 B).

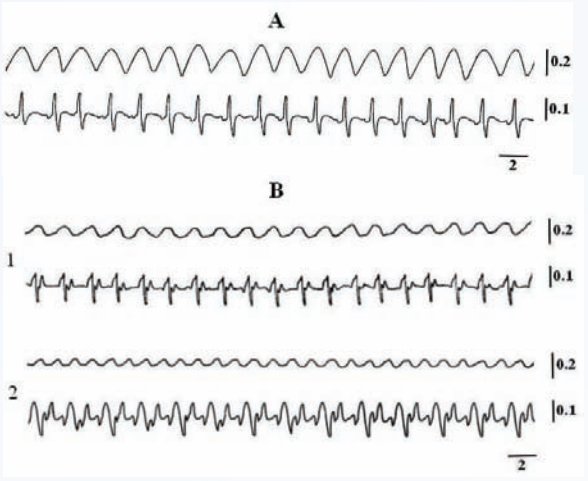

***Figure** 1. The effect of histamine on the slow-wave and spike activities of the ureteral perirenal zone.*
***A:** normal conditions;*
***B:** under the action of histamine (10^{-4} Mol/L): 1) increase of activity; 2) increase of activity and regulation of spike forms. N=12. Calibration: amplitude (mV), time (C).*

Improvement of conductivity is also recorded and each spike that occurs on the base of a slow wave, as a rule, reaches the peribladder zone. Along with an increase of automaticity of area bordering to pyeloureteral anastamosis, in less than half of the cases, 37±3.2% (n = 9), an increase in duration of action potentials genesis is observed, similar to earlier obtained results in the ureter of guinea-pigs [*Kazaryan K. et al., 2003*] (Figure 1 B 2).

In the next series of experiments we studied the effect of histamine on the spontaneous activity of distal part of the ureter and bordering area of the bladder. Figure 2 A shows slow-wave oscillatory process in norm along with the action potentials in this area of the bladder and spike-form excitatory waves propagating from the parirenal area. In this case the latter are imposed on different phases of slow waves of peribladder zones (Figure 2 A 2; shown by the arrow). If in the perirenal area the appearance of spikes completely correlated with the slow-wave activity, then for the peribladder zone of the ureteral distal part such coordination with automatism of the bladder bordering zone is missing. However, when comparing the moment of spikes occurrence in the studied area of urinary bladder with phases of slow oscillations in the neighboring ureteral zone a certain relationship between these types of activity is noted: spikes correspond only to definite phases of slow waves. Thus, according to Figure 2 A 1, action potentials correlate with the ascending or descending phases of the waves.

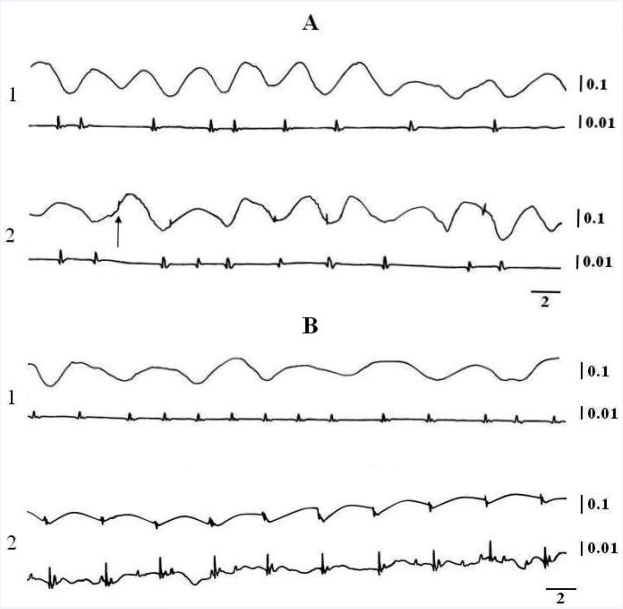

FIGURE 2. The effect of histamine on the slow-wave activity of the ureteral peribladder zone and spike activity of the bordering bladder zone.
A: *normal conditions: 1)* correspondence of spikes with slow oscillations phases; 2) imposition of propagating *action* potentials of renal area on the slow waves.
B: under the action of histamine (10^{-4} Mol/L): 1) reduction of slow-wave frequency and increase in the frequency of spike activity; *2* - the effect of action potentials propagating from the renal area. *N* = 12. Calibration: amplitude (mV), time (*C).*

A different type of correlation is also possible at which the coherence of spikes genesis with other phases is observed, as shown in Figure 2 A 2: either with the lowest point of the wave, or its crest. The injection of histamine leads to certain changes of spontaneous activity. In contrast with the area of pyeloureteral anastamosis the frequency of slow-wave activity of the distal part of the organ is slowed, while fluctuations are stretched and become irregular. The frequency of spike activity in these conditions increases by 56 *oscill/min*, without violating the described higher correlation with the waves (Figure 2 B 1). The above-mentioned improvement of conductivity of ureter entails the propagation of all spikes in more than half of the cases (55-60%, n = 12) not only to ureteral anastomosis, but also to the bladder (Figure 2 B 2). Inhibition of slow waves and disturbance in rhythm of coordinated spike activity is noted.

Figure 3 shows the changes in frequency characteristics of both types of activity, which is connected with changes of injected histamine concentration (10^{-6}-10^{-4} *Mol*/L) for all the studied regions. Since the frequency of spike and slow-wave ac-

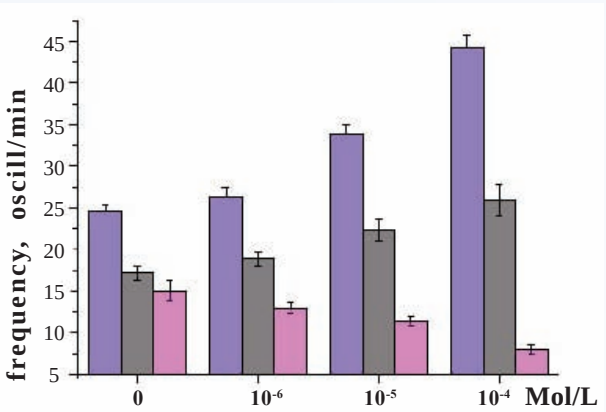

FIGURE 3. The frequency of spontaneous activity *in* different parts of the ureter and upper zone of the urinary bladder depending on histamine concentration.
slow wave and spike activity of perirenal zone
spike activity of urinary bladder
slow wave activity of peribladder area

tivities of renal area coincide, there are single columns for them. Increasing concentration of histamine up to 10^{-4} *Mol/L* leads to a gradual increase in the frequency of spike activity for proximal area of the ureter (from 24.5±2.6 to 44.3±4.2) and the upper zone of the bladder (from 18.2±1.5 to 25.9±2.8 *oscill/min*), respectively (Figure 3). In the ureteral peribladder zone a decrease in frequency of slow-wave activity is marked.

Presented electrophysiological studies have confirmation in the next series of experiments conducted by the morphohistochemical method.

It is shown that due to identification of orthophosphates [*Meliksetyan I. et al., 1990*] on the frontal sections of the ureter, cellular structures were revealed throughout its length. Outside, directly adjacent to the suburotelium there is a muscular layer, where the number of longitudinal and circular muscle layers varies in different ureteral regions (Figure 4 A; F 3). Myogenic structures of perirenal area of the ureter (Figure 4 A 3) are detected most intensely. The myogenic structures in the wall of ureteral peribladder zone are clearly distinguished, but in this area myogenic elements

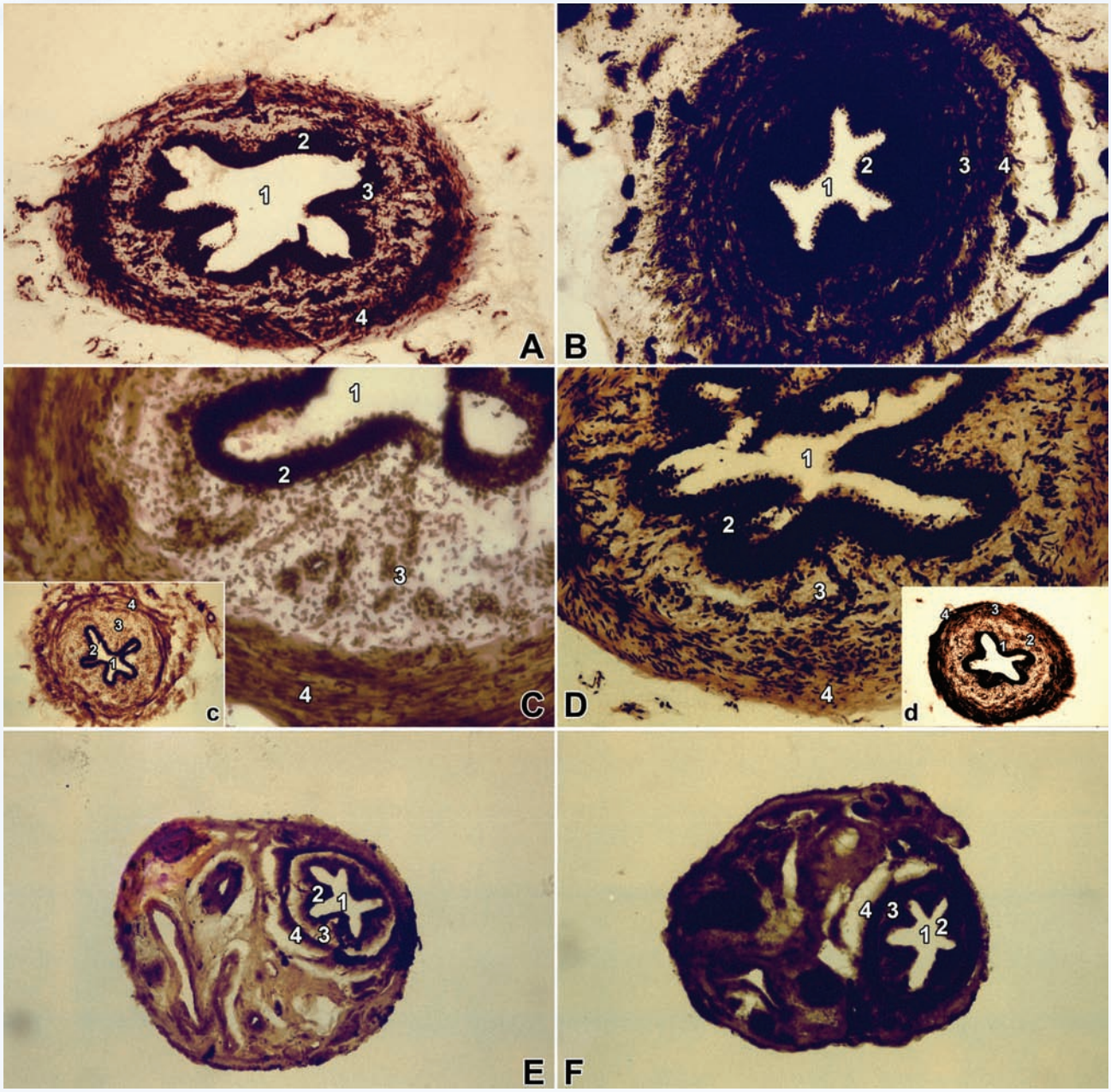

FIGURE 4. *Frontal sections of ureteral perirenal area (A), ureteral peribladder area (C), bladder (E) of intact rats and perirenal area (B), peribladder area (D), bladder (F) under the action of histamine (1: lumen of the ureter; 2: mucosa; 3: muscular sheath; 4: adventitia). Method of orthophosphates detection. Magnification: A, D - 160; B -1000; b, e; C, F - 63; E - 400.*

are evenly spaced with a moderate degree of intensity (Figure 4 C 3).

Under the influence of histamine in all regions of the ureter and urinary bladder increasing activity of phosphates identification was clearly observed. In perirenal area the phosphate activity is so enhanced that it creates impression of diffuse darkened staining (Figure 4 B). In the ureteral peribladder area (Figure 4 D) and in the urinary bladder (Figure 4 F) under the action of histamine myogenic elements are clearly identified, but in comparison with intact animals phosphate activity increased.

Thus, these morphological and histochemical studies showed that the muscle membranes have considerable differences in metabolism of myogenic elements and myogenic structures near the renal pelvis of the ureter both in intact rats and those under the effect of histamine were the most intensely stained.

DISCUSSION

The investigation of automaticity of the ureter directly adjacent to the bladder has revealed spontaneous activity of both slow-wave and spike electrical oscillations, the frequency of which is much lower than those for perirenal pacemakers: spike activity more than three times less, and slow-wave – up to two times. At the same time, in norm we did not find coordinated operation of rhythmogenesis in the ureteral distal area with the main pacemaker activity localized in the pyeloureteral anastomosis. However, rhythmogenesis of peribladder zone under normal conditions was consistent with the spike activity of ureteral bordered area with bladder. Presented data on the effect of histamine has revealed a possible correlation between pacemaker activity of the two edging zones of the rat ureter.

According to the earlier studies, if in normal conditions central part of the ureter is a conductor of activity between the two initial pacemaker regions, the influence of histamine promotes identification of rhythmogenic characteristics of the area [*Santicioli P. et al., 1998; Kazaryan K. et al., 2001*]. The impact of this drug on the activity of ureteral smooth muscle tissue is expressed by the regulation of both types of pacemaker activity (slow-wave oscillations of membrane potential and spike activity) and the nature of rhythmogenesis [*Shuba M., 1977; Kazaryan K. et al., 1999*]. Moreover, under the effect of histamine the improvement of the patterns of activity is also recorded, as well as the acceleration of spike activity generation in relation to background slow oscillations of membrane potential [*Kazaryan K. et al., 1995*]. Indeed, as shown in this study, histamine contributes to spreading of spikes to the bladder, inhibiting at the same time the slow-wave activity of peribladder zone. Under these conditions, marked segment of the ureter becomes a conduit of activity for propagating electrical wave and thus co-ordinates with the main perirenal rhythmogenesis.

As shown above, under the action of histamine myogenic structures near the renal pelvis, in contrast to the distal zone of the organ, are stained so intensely that it creates the impression of diffuse darkening. Such high functional activity of renal pacemakers avails propagating spikes to the bladder and may cause suppression of rhythmogenesis of ureteral peribladder zone in these conditions.

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