Urol Res (1992) 20:173–176

Urological Research

Analysis of the detrusor smooth muscle action potential

O. M. A. Karim, N. Seki, and J. L. Mostwin

James Buchanan Brady Urological Institute, Johns Hopkins Medical Institutions, Baltimore, Maryland, USA

Accepted: October 1, 1991

Summary. A method is described to record bladder smooth muscle action potential (AP) data and subsequently in digitized form analyze the constitutive elements of the AP. Manipulation of digitized data can give accurate descriptive information on the AP configuration and kinetics. In the future this type of analysis will hopefully lead to more precise, quantitative information on changes in the smooth muscle AP kinetics with disease states and facilitate a clearer understanding of the pathophysiological processes underlying changes in detrusor contractility.

Key words: Bladder – Depolarization – Repolarization – Membrane potential – Contractile property

The action potential (AP) is the electrical membrane event most associated with muscle cell signalling activity and has been shown to play a significant role in regulating tonic and phasic changes in bladder contractility [8]. Impaired contractility has been shown to be a feature of the detrusor muscle from hypertrophied bladders secondary to experimentally induced outflow obstruction [2, 7, 13]. In the cardiovascular system, a decreased maximal shorting velocity of hypertrophied venous smooth muscle has been documented [1]. In the pressure-overloaded myocardium, significant electrophysiological changes occur in the hypertrophied myocardium, implying that structural changes are associated with changes in AP properties [4].

In contrast to the cardiovascular system, very little work has been done to categorize the electrophysiological changes in the bladder smooth muscle with disease states. In view of the changes seen in the hypertrophied myocardium [3, 4], it might be reasonable to suspect that similar changes occur in the hypertrophied detrusor smooth muscle. Before, however, any statements can be made about disease-induced changes in the bladder smooth muscle AP, there must be standardization of terminology. In particular, the duration and kinetics of the AP need to be clearly defined, since these properties are most effected with desease in the cardiovascular system [3, 5, 6, 9, 10, 12]. As yet, there has been no recommended protocol for measuring the detrusor smooth muscle AP in the normal or diseased state. The purpose of this paper is, thus, twofold: firstly, to report a method by which detrusor smooth muscle AP parameters can be measured with reproducible accuracy; and secondly, based on these observations to suggest standardization of measurement of AP duration from the point of maximal depolarization.

Materials and methods

Tissue preparation

Bladders were removed from male albino guinea pigs (Hartley Strain, Charles River) weighing 400–600 g, which were sacrificed by CO_2 inhalation. Mucosa-free detrusor was prepared by careful removal of the mucous membrane and submucosa under binocular vision using a dissecting microscope. Parallel, longitudinal muscle bundles (10 mm long × 2–3 mm in width) were selected for microelectrode study. Muscle strips were placed, mucosal side uppermost, in a recording chamber (2 ml capacity) and superfused with warmed (35–36°C) Krebs solution [composition (mM): Na^+, 137.0; K^+, 5.9; Ca^{2+}, 2.5; Mg^{2+}, 1.2; HCO_3^-, 15.5; $H_2PO_4^-$, 1.2; Cl^-, 134; glucose, 11.5] at a constant flow rate of 2–3 ml/min. The Krebs solution was bubbled continuously with 95% O_2/5% CO_2, and the pH of the solution was maintained at 7.2–7.3. The solution was made hypertonic by addition of 12 g sucrose to 100 ml Krebs solution in order to reduce tissue movement [11]. Following equilibration (1.5 h), the muscle strip was immobilized with tiny pins on a silicon rubber plate (KE-66, Shin-Etsu Kagaku, Tokyo, Japan) which covered the bottom of the chamber. Electrical responses of the bladder smooth muscle cell membrane were recorded using glass capillary microelectrodes (1.2 mm outer diameter) filled with 3M KCl. The tip resistance of the electrodes ranged from 40 to 60 MΩ.

Data collection and storage

The spontaneous APs recorded from the smooth muscle membrane were displayed on a cathode-ray oscilloscope (Tektronix 5113). A real-time hard copy of the APs was made using a pen-writing

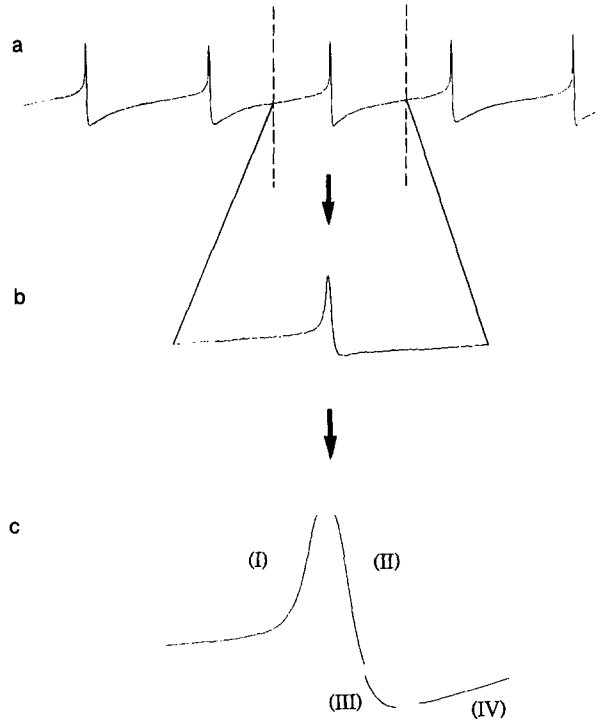

Fig. 1 a–c. Schematic representation of how the action potential is split into its constitutive components. **a** *Vertical dotted lines* represent cursors positioned symmetrically, either side of the action potential to be analyzed, approximately equidistant to two spike potentials. **b** Indices of the vertical cursors allow the action potential data to be extracted as a subarray. **c** Using the indices of the maximum and minimum potential values, the resting membrane potential value and the boolean operators >=, the action potential can be split into (I) depolarization (II) repolarization, (III) hyperpolarization and (IV) after polarization segments

recorder (Gould 220) and data were simultaneously digitized through an analog to digital converter (Data Acqusition A/D converter AT-Codas Waveform scroler, Dataq Instruments) at a sample rate of 2 kHz (2000/s) and stored on magnetic disc (Dell 316 SX) for subsequent analysis. Data files generated from a typical tissue preparation were approximately 3–5 Mb in size; hence, for analysis, the relevant segments of AP data (5–7 APs at a time) were exported in a file format compatible with our analysis software.

AP analysis

The AP analysis program was written in ASYST™ (Asyst Software Technologies) a scientific programming high-level language which is particularly suited for waveform analysis. A particular attribute of this language is that it is a stack-based language, placing indexed data arrays on a numerical data stack. Individual or multiple elements of an array can be referenced and subsequently subset arrays of predetermined indices produced. A waveform scroller mode in the software allowed compression and expansion of the small 5–7 AP files. Vertical cursors were positioned either side of the AP to be analyzed and, using the cursor indices, a subarray containing the relevant AP was generated (Fig. 1a, b). The points of the maximum and minimum potential values were indexed and saved as the overshoot potential (OS, mV) amplitude and after hyperpolarization (mV) values, respectively. The resting membrane potential (RMP, mV) was the mean of the potential values of the cursor points defining the start and end of the AP. The AP amplitude

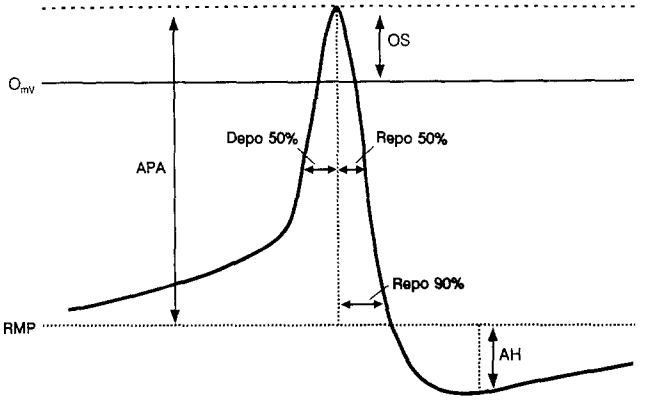

Fig. 2. Details of measured action potential parameters. Action potential amplitude (*APA*, mV), overshoot (*OS*, mV), after hyperpolarization (*AH*, mV), resting membrane potential (*RMP*, mV), action potential duration, measured as time to 50% depolarization (Depo 50%, ms) or 50% (Repo 50%, ms) and 90% (Repo 90%, ms) repolarization. The maximum velocity of depolarization (dv/dt, Vs^{-1}) and repolarization (dr/dt, Vs^{-1}) are the maximum values of the first derivatives of segments (I) and (II) respectively in Fig. 1c

(APA, mV) was calculated as the difference between OS and RMP (Fig 2).

The indices of the maximum and minimum potential values in the AP array served to subdivide the AP array further into depolarization, repolarization/hyperpolarization and after polarization segments. The repolarization/hyperpolarization segment was further split into repolarization and hyperpolarization segments using the boolean operators ">=" (greater than or equal to) the RMP (Fig. 1c). Using electronic differentiation of the depolarization and repolarization segments, the maximal values of the first derivatives gave the maximal value of AP depolarization (dv/dt, VS^{-1}) and AP repolarization (dr/dt, VS^{-1}), respectively.

The AP duration was expressed as the time taken to reach predefined events in the depolarization and repolarization segments of the AP. The time to 50% depolarization (Depo 50%, ms) was the time from 50% depolarization to the point of maximal depolarization. Similarly, the time to 50% (Repo 50%, ms) and 90% (Repo 90%, ms) repolarization were the times taken to reach 50% and 90% repolarization following the point of maximum depolarization (Fig. 2). The amplitude of the membrane potential at these respective points was calculated and using the boolean operators ">=" the indices of these points in the depolarization and repolarization segment subarrays could be determined. Using the index of the maximal potential as the reference point the times taken to reach the predefinde events in the AP could be calculated from the product of the difference in the indices of the respective points and the sample rate (2 kHz or 0.005 s between points). All calculations and AP values were in the computer random access memory (RAM) and following completion of the analysis, exported to a spreadsheet.

The values recorded were expressed as mean ± the standard deviations (SD).

Results

The results of the AP parameters recorded from normal detrusor smooth muscle membrane are given in Table 1. The results indicate that there is some symmetry of the spike potential with similar values for durations of the Depo 50% and Repo 50% either side of the point of maximum depolarization. The values calculated for the maximum velocities of depolarization (dv/dt) and repo-

Table 1. Action potential parameters

Number	APA (mV)	AH (mV)	OS (mV)	Depo 50% (ms)	Repo 50% (ms)	Repo 90% (ms)	dv/dt (V/s)	dr/dt (V/s)
$n=36$	49.7 ± 3.1	12.7 ± 3.5	10.5 ± 3.1	5.8 ± 0.6	4.7 ± 0.8	7.2 ± 1.3	6.3 ± 0.9	9.4 ± 1.6

Values are mean ± *sd*, from normal guinea pig bladder smooth muscle. APA, action potential amplitude; AH, after hyperpolarization; OS, overshoot potential; action potential duration expressed as time to 50% depolarization (Depo 50%) or 50% (Repo 50%) and 90% (Repo 90%) repolarization; dv/dt velocity of depolarization; dr/dt velocity of repolarization

larization (dr/dt), similarly, reflect the Depo 50% and Repo 50% values respectively, with a shorter Repo 50% correlating with the increased velocity of repolarization. From the data, the RMP, calculated as the differences between APA and OS, was approximatly 39–40 mV, which is in agreement with previously published work [8]. Once recorded on magnetic disc, the values of each digitized point of the AP did not change. Repeated analysis of the same AP with the software, therefore, gave the same measured parameters (not shown).

Discussion

The biphasic nature of the detrusor smooth muscle AP makes it particularly suited to waveform analysis. Using commercially available programming software and computer hardware readily found in most bladder muscle physiology laboratories undertaking electrophysiological work, we have demonstrated how AP data can be stored and subsequently analyzed. Capitalizing on the biphasic nature of the AP, we have demonstrated how manipulation of digitized data can split an AP into its constitutive parts and give accurate descriptive information on the AP configuration and kinetics.

The duration of the AP spike potential is approximately 15–20 ms. We chose a sample rate of 2 kHz which generated large data files and gave us 30–40 sample points during the spike phase of the AP. This sample rate was, thus, sufficient for our purposes and allowed us to differentiate the depolarization and repolarization segments with reproducible results. There may be advantages in increasing the sampling rates, e.g. in neuronal tissue; this, however, would be at the expense of computer hard disc memory. A potential disadvantage of decreasing the sample rate excessively must be noted when dealing with digtized data in that with the rapidity of the AP spike it might be possible to miss the true maximum of minimum potential values.

We chose to describe the AP duration as the time taken to reach predefined AP events. This concept, though new to bladder muscle electrophysiologists, is established in the cardiovascular system [4]. The index of the maximum spike potential, unlike the slow membrane depolarization prior to the onset of the AP, in digitized data is a finite point and, hence, the ideal reference point for timing AP events. In our opinion, the Depo 50%, Repo 50% and Repo 90% values we chose give a good characterization of the AP shape and appear to complement the data on the kinetics of the membrane potential in their respective segments.

The use of boolean operators to determine the point at which the repolarization curve intersects the RMP or to measure the Depo 50%, Repo 50% and Repo 90% values might initially appear to be introducing error. With a sample rate of 2 kHz, the error (x), in calculating a time (t) would be $t \pm x$, where $0 < x < 0.0005$ s. The value of x would decrease wilth an increase in sample rate such that $x = 1/\text{sample rate}$.

In conclusion, we have demonstrated an accurate, reproducible way to analyze the detrusor smooth muscle AP. This type of analysis will hopefully yield more quantitative descriptive information on changes in the smooth muscle AP kinetics with desease states and facilitate a clearer understanding of the pathophysiological processes associated with changes in detrusor contractility.

Acknowledgements. This work was supported by funding from PHS grants D538466-03 and AM19300. Mr. Omer M. A. Karim was supported by a King Edward VII Hospital Fund Travel Bursary and an Ethicon Foundation Travel grant from the Royal College of Surgeons of Edingburgh.

References

1. Arner A, Malmqvist U, Uvelius B (1985) Effects of Ca^{2+} on force-velocity characteristics of normal and hypertrophic smooth muscle of the rat portal vein. Acta Physiol Scand 124:525
2. Arner A, Malmqvist U, Uvelis B (1990) Metabolism and force in hypertrophic smooth muscle from rat urinary bladder. Am J Physiol 258:C923
3. Aronson RS (1980) Characteristics of action potentials of hypertrophied myocardium from rats with renal hypertension. Circ Res 47:443
4. Cameron JS, Myerburg RJ, Wong SS, Gaide MS, Epstein K, Alvarez RT, Gelband H, Guse PA, Bassett AL (1983) Electrophysiologic consequences of chronic experimentally-induced left ventricular pressure overload. Am J Cardiol 2:481
5. Coltart DJ, Meldrum SJ (1970) Hypertrophic cardiomyopathy: an electrophysiological study. BMJ 4:217
6. Gelband H, Bassett AL (1973) Depressed transmembrane potentials during experimentally induced ventricular failure in cats. Circ Res 32:625
7. Mattiasson A, Uvelius B (1982) Canges in contractile properties in hypertrophic rat urinary bladder. J Urol 128:1340
8. Mostwin JL (1986) The action potential of guinea pig bladder smooth muscle J Urol 135:1299

9. Ten Eick RE, Gelband H, Kahn J, Bassett AL (1977) Changes in outward transmembrane currents of papillary muscle of cats with right ventricular hypertrophy (abstract). Circulation 56:47
10. Tholon C, Kreher P (1989) Altered electrical response to caffeine exposure in hypertrophied rat myocardium. Can J Physiol Pharmacol 67:1471
11. Tomita T (1966) Electrical response of smooth muscle to external stimulation in hypertonic solution. J Physiol (Lond) 183:450
12. Tritthart H, Leudcke H, Bayer R, Stierle H, Kaufmann R (1975) Right ventricular hypertrophy in the cat: an electrophysiological and anatomical study. J Mol Cell Cardiol 7:163
13. Van Koeveringe G, Mostwin JL, Mastrigt R van, Van Koeveringe BJ (1990) Effect of partial obstruction on force development in guinea pig bladder muscle (abstract). J Urol 143:355A

Mr. Omer M. A. Karim, FRCS, FRCSE
Research Fellow in Urology
Institute of Urology and Nephrology
172 Shaftsbury Avenue
London WC2
United Kingdom