Studies on the Action Potentials, Accompanying Spontaneous Peristalsis of Cat's Ureter, *in situ.**

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The monophasic action potentials of mammalian ureter were successfully recorded by E. Bozler in 1942[2]. Since then, many investigations on the same material, excised or *in situ*, have been reported with the refined amplifing and recording apparatus.

On the other hand, the method of intracellular potential recording made it possible to investigate the electrophysiological character of nerve- and cardiac cell, by using the membrane potential as a parameter. Similar investigations on a smooth muscle cell have been performed along this line, with a little success. As for a ureter-cell, however, no successful investigation has been achieved hitherto.

Since the pattern of the surface action potential of the ureter varies according to the electrode position or to the electrode distance[2], monophasic potential recordings are required for elucidating the electrophysiological nature of the ureter. And, its precise recording was attempted in the present study, using monopolar lead and suction electrode or glasscapillary one.

MATERIALS and METHODS

All experiments were carried out on the exposed cat ureter, *in situ*, and its action potentials accompanied with spontaneous peristalsis were investigated. Laparatomy was performed under pentobarbital-Na anesthesia (25 mg/Kg, intraperitoneal). The intestines were pushed aside by using surgical hooks and covered with wet gauze previously immersed in Tyrode's solution (38°C). The peritoneum and the connective tissue over the ureteral part, where the exploring electrode would be placed, were carefully removed. The room-temperature was between 28 and 32 (C). It was not controlled to the body temperature, because the disturbing effect of the shivering was not seen during the experimental procedure (1–2 hours).

As an exploring electrode, ordinary Ag-AgCl-electrode, suction electrode or glasscapillary one was used. It was prepared with a slight modification of the usual pro-

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cedure, and the details of this modification were written below in the respective section. The indifferent Ag-AgCl electrode was placed in the fatty tissue, apart more than 1cm from the ureter. In this position the electrotonic spread of ureter action potentials was negligible.

A cathod follower amplifier with a well selected 1/2 12AU7 as head tube was used, whose output was amplified and recorded by both cathode-ray oscilloscope and ink-writing electromagnetic one.

RESULTS and DISCUSSION

1. *Surface Electrogram*

The upper and middle records (A and B) in Fig. 1 were obtained by monopolar, and lower one (C) by bipolar lead. The cotton thread moistened with Ringer's solution (so-called "wick-electrode") was used as an exploring electrode in A. In B and C, ordinary Ag-AgCl electrodes were used. Downward deflection in the monopolar record showed the negativity of the exploring electrode (same, in the following figures).

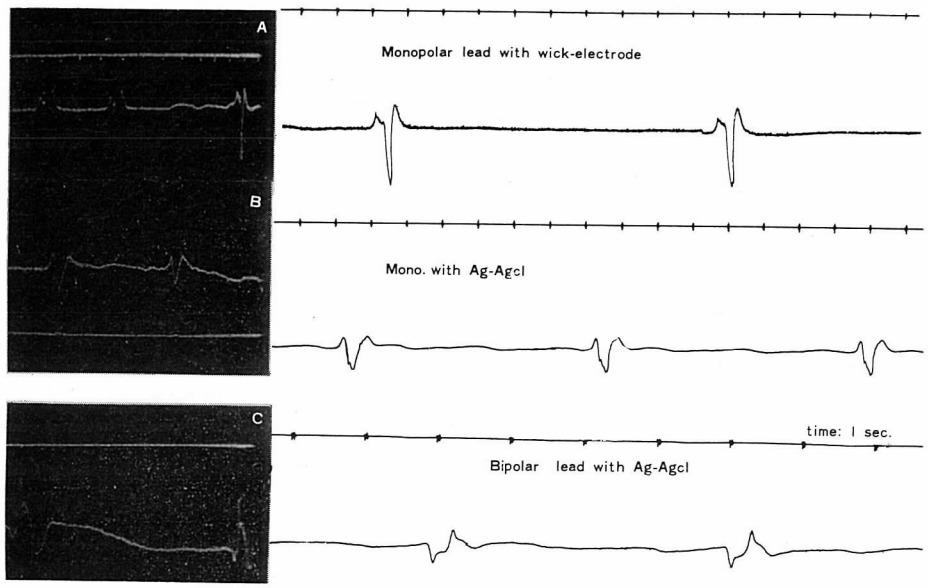

Fig. 1. Surface electrograms of cat's ureter, in situ, accompanyed with spontaneous peristalsis.
A and B: Monopolar leads with wick-electrode and Ag-AgCl electrode, respectively. Downward deflection shows the negativity of the exploring electrode.
C: Bipolar lead with Ag-AgCl electrodes, distance of which was 10 mm.
In each row, left-handed photograph shows the record by cathoderay oscilloscope and right handed, by inkwriting electromagnetic one with the same preparation. Arbitral amplification, time scale, 1 sec.

The monopolar action potential was three-phasic; i.e. first positive-, second negative- and the last positive-waves were recognized in the recordings. The configuration was same as that in some reports of other investigators[16, 18]. A pattern of the bipolar action potential, on the other hand, was poliphasic and showed various forms according to the distance of the leading electrodes. It had been discussed fully[9] that the configuration of the bipolar action potential could be interpreted as an algebral summation of the monopolar action potentials at each electrode. This interpretation was thought to be applicable in the present study and the discussion concerning the pattern of bipolar action potential was laid aside. Other experimental findings, reported hitherto, such as the decreases of the frequency and the conduction velocity of peristaltic wave and/or the prolongation of action potential duration were also observed at the low experimental temperature (less than 38°C).

It had been reported that the first positive wave in the monopolar recording was the field-effect caused by the coming impulse to the cell-groups near the exploring electrode. That is, in this moment, the ureter cells around the exploring electrode were not active but effective as a "source" for the local potential recording. In other words, the cell-groups on which the exploring electrode was placed were not active and the recorded potential variation was caused by the activity of neighbouring cells in the so-called "sink" area. This interpretation might be accepted in the present experiment.

The second negative wave was thought to be the main action potential of the cells around the exploring electrode. Its amplitude or time-course was varied by the experimental conditions, such as moistening degree of the preparation, way of placing the electrodes, time elapsed after the operation or experimental room-temperature in which the recording was done. The hump seen at the downward deflection in Fig. 1–B might be interpreted as showing the asynchronous activity of the ureteral cells from which the action potential was recorded.

The explanation of the last positive wave had been incompatible. H. Niu[9] interpreted it as the field-effect caused by the foregoing impulse. On the other hand, R. Kudo[13] reported that the potential variation might be caused by the coming impulse to the indifferent electrode, placed at the distal (from the kidney) inactive part of the ureter, refering the E. D. Adrian's work[1]. Concerning this explanation, Fig. 2 might be consulted.

In the upper half of Fig. 2, the bipolar action potential was illustrated for a comparison. The Ag-AgCl electrode was bent so as to take a hook-like shape. It was inserted under the ureter which was slightly lifted up after the procedure.

Later from the recording of the upper figure (Fig. 2–A), the ureter was ligatured between the two electrodes and the lower figure was recorded. Both bipolar and monopolar action potential could be seen in this record. The interpretation of the phenomena was such that the conduction of the impulse was blocked at the ligatured area in one case but not in the another. If Kudo's interpretation stated above

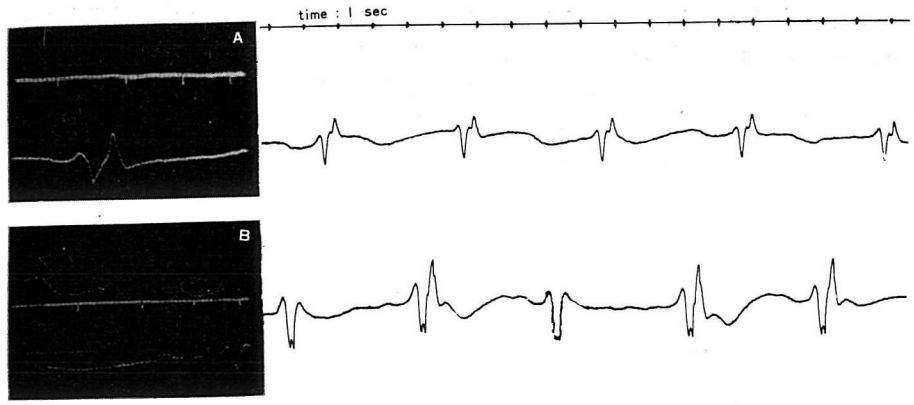

Fig. 2. A: Bipolar surface electrogram, for a control, with the same procedure as Fig. 1-C.
B: Records where the ureter is ligatured between the two electrodes. Note that both monopolar and bipolar electrogram are recorded.
(with arbitral amplification and the time scale written in the figure.)

were applicable, the last positive wave would not be recorded in the monopolar action potential. Because, the impulse from renal side was blocked at the ligatured part and was not conducted to the distal electrode. The wave, however, could be seen clearly in Fig. 2. Therefore, it was reasonable to interpret it as a field-effect according to Niu and the others.

By the way, the electrograms from the human ureter using the bipolar electrode attatched to the catheter had been reported by Kneuker[12]. The reported electrograms were very irregular. It was thought that the potential variations had not a clear distinction from the artifacts and that they did not show the action potentials of the renal cell as he had insisted.

2. *Recording with suction electrode*

Using a suction electrode, the monophasic action potential has been reported by other investigators[7, 9] on the cardiac muscle of frog or of oyster. Similar studies were attempted in the present experiment.

The sketch of the suction electrode was presented at lower right in Fig. 3. Its one type was glass syringe (1–2 ml). The tip of needle was ground and gilded with Ag-AgCl. Another type was made of electrode-tip (exchangeable small pipette), connecting tube, three-way glass tube and mercury manometer. The leading silver wire was inserted to the tip through the three-way tube. The suction was attained by lowering the mercury bulb and the resulted negative pressure was read on the manometer.

It was preferable to make the dead space, which was not filled with mercury, as scanty as possible in order to attain a good suction. The length of the connecting tube could be adjusted according to the experimental condition. Perhaps, this type

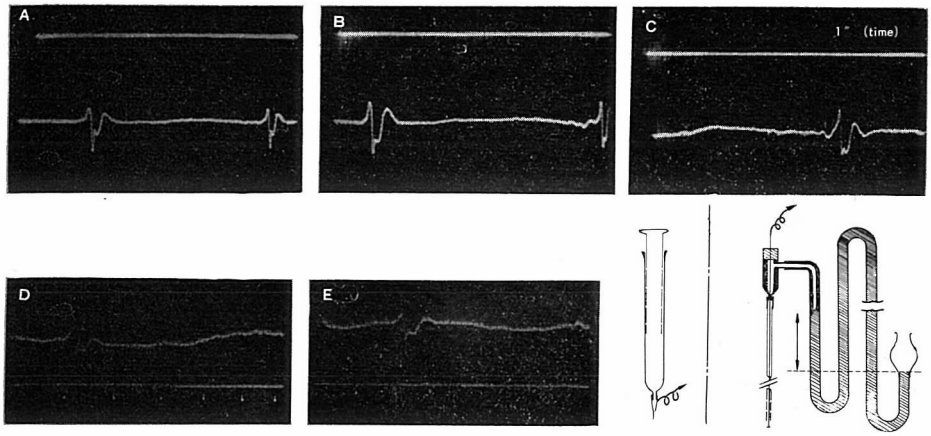

Fig. 3. Two monopolar recordings obtained through the suction-electrode.
Lower right: Sketch of the electrode.
A & D: records before the suction for a comparison (surface electrogram).
B & E: after the suction.
C: another comparison or the recovery. The suction was released after the recording of B.
(Amplification and time scale: same as Fig. 2 or Fig. 1)

of electrode would be applicable to the clinical usage if the ureteral catheter were used as the connecting tube and the good fixation of the tip to the ureteral wall could be achieved.

Two recordings obtained through the suction electrode were represented in Fig. 3. In this figure, both in upper and lower, left ones (A and D) were recorded before the suction and middle ones (B and E), during it (negative pressure, 5–10cm Hg). After the recording of Fig. 3–B, the suction was released and Fig. 3–C was obtained 10 minutes later. It was reasonable that the action potentials before the suction and after the release of it had the similar configurations with those of monopolar recording. The expected monophasic potentials, however, could not be recorded by the suction.

Generally speaking, in order to record monophasic action potential through a suction electrode, the local demarcated injury might have to be caused by the suction at the area on which the electrode-tip is placed. The negativity of the exploring electrode seen at the beginning of the suction, if any, would be the result of this injury. The monophasic action potential would arise from the negative baseline and be directed positively. Therefore, the recorded action potential might show the activity not of the cell-group in the injured area but of that in the adjacency. The above stated interpretation on the first positive wave in the monopolar recording might also be applicable in these cases.

In Fig. 3, however, the recorded action potential was not monophasic but mono-

polar. This might be caused by the condition that there was not any injury as a result of insufficient suction. Only in few cases, the monophasic records were obtained, but it was not reproducible. The histological difference between the cardiac tissue and ureteral one would be another cause: because, in the preliminary experiments on the frog's heart, it was easy to obtain the monophasic action potential through the same electrodes as those shown above. The study of this difference, however, was beyond the scope of the present experiment.

3. *Recording with glass-capillary electrode*

The glasscapillary electrode (synonim, microelectrode or intracellular electrode) was prepared by using KATSUKI-type puller and was filled with 3-molar KCl solution[10,11]. It was flexibly mounted on the preparation according to the device of Woodbury et al.,[22] or attached to a half rigid holder on a micromanipulator: and the insertion of the electrode-tip into the ureteral muscle-cell was attempted.

An experiment to record the intracellular monophasic action potential through these procedure was without success; and the monopolar three-phasic action potentials were almost always observed. Therefore, the method to prepare the exploring electrode was slightly modified. The glass capillary made by the puller were artificially broken at the tip and those which had the outside tip-diameter between 40 and 150 microns were selected. They were filled with Ringer's solution, instead of KCl. The recording obtained with such an electrode was represented in Fig. 4.

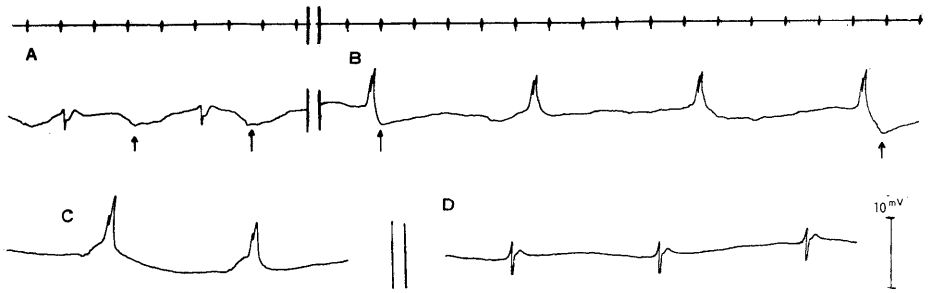

Fig. 4. Recordings with the glass-capillary electrode which has a large tip-diameter and is filled with Ringer's solution. (Details are in the text)

A: Monopolar surface electrogram for a comparison.

B & C: Monophasic action potential recorded when the electrode-tip was inserted into the ureter.

D: Another comparison. The electrode was pulled out to the surface after observing A, B and C.

The deflections indicated by the arrows are the respiratory artifacts.

Figure 4–A (upper left) was recorded by placing the electrode on the ureter and this was the monopolar surface electrogram for a comparison. The electrode-tip was then thrusted into the ureteral tissue and Fig. 4–B (upper right) was obtained.

The configuration of action potential was not changed for 1.5 hours after the insertion. Its amplitude, however, decreased gradually. After these observations, the electrode was slightly drawn back and Fig. 4–C (lower left) was recorded. The decreased amplitude was restored to the level at the beginning of 4–B. Thereafter, the electrode was pulled out from the inside of the tissue to the surface and Fig. 4–D (lower right) was obtained. The negative slow waves shown by the arrows were the artifacts caused by the respiratory movement of the animal. The tip-diameters of the electrode were 79 microns at its inside and 112 microns at the outside.

It had been reported by Y. Takeo[21], my collaborator, that the monophasic action potential could be recorded by inserting the Ag-AgCl needle-electrode into the cat's heart, *in situ*. Apart from the other experimental procedures, it was noteworthy that his exploring electrode could not give the intracellular potential variations and that the reported monophasic action potential was not the intracellular, but the intercellular one. Analogously, the record in Fig. 4–B or 4–C had to be considered as showing the intercellular action potential, because the tip-diameter of the exploring electrode was large and/so it could not pick-up the intracellular potential. The upward deflection and the hump in these cases could be interpreted as stated above; i.e. the former showed the activity of the not-injured cell-group around the inserted electrode-tip and the latter was resulted from the asynchronization of this activity. The amplitude would be determined by the electrotonic spread of the action potential of the cell-group. The gradual decrease of it might be caused by the cell-inactivation which would spread out from the electrode-tip into the farther area, as the time elapsed after the insertion. The observation in Fig. 4–C, where the re-establishment of large amplitude was attained by the slight dislocation of the tip at the time when the amplitude had become smaller, would be a support of this assumption.

The record in Fig. 5 was obtained through the electrode whose tip was also artificially broken. The KCl solution, however, was filled in comparison with Fig. 4.

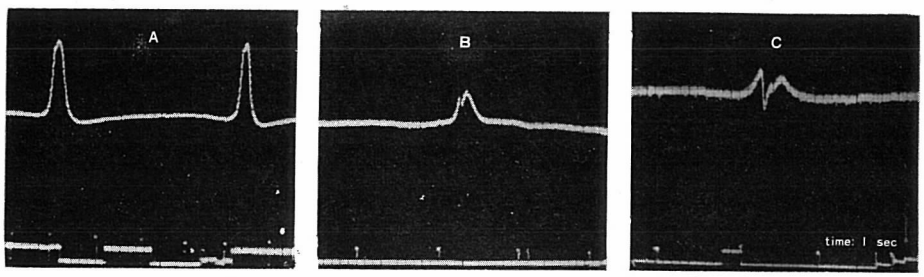

Fig. 5. Recordings with the glass-capillary electrode which has a large (30 microns, outside) tip diameter and filled with 3-molar KCl solution. (See in the text)

A: immediately after the insertion. B & C: 20 and 30 minutes later, respectively.

Its outside diameter was about 30 microns. Figure 5–A was recorded 2 minutes after the insertion; B and C were, 20 and 30 minutes, respectively.

In Fig. 5, the configuration of the action potential and its deformation after the insertion were similar with those in Fig. 4. Going into detail, however, slight differences were noticed: the monophasic feature was more conspicuous and the larger amplitude was easily obtained in the former at the beginning of the insertion. Also in the former, both the gradual decrease of the amplitude and the deformation from monophasic to three-phasic shape were faster.

The difference of experimental method between Fig. 4 and Fig. 5 is found in the exploring electrode. One was filled with Ringer's, and the other with KCl solution. The potassium-ion, as is well known, has an ability to depolarize the excitable membrane and/so to inactivate the muscle cell. The electrodes used in both recordings have large tip-diameter and the solution in it would easily leak out. It may be assumed in Fig. 5 that the injury were demarcated in the small area at the beginning of the insertion. The relatively larger amplitude and the shape of action potential having more monophasic feature in the Fig. 5, may be interpreted from these considerations. In addition, the spreading out of potassium-ion from the electrode-tip into the neighbouring cells is very rapid owing to its diffusing ability, and the faster decrease of the amplitude or the relatively faster deformation of the configuration, also in Fig. 5, may be reasonable.

A glass-capillary electrode was introduced by Ling and Gerard[14] in 1949 as a technique for intracellular potential recording. The criteria to it were reported by Nastuck and Hodgkin (1950) on a frog's sartorius muscle[15] or by Bülbring and Hooton (1954) on a rabbit's sphincter pupillae[3]. According to them, a potential recording can be approved as an intracellular one in the case where the following findings are recognized: (1) The negative deflection is suddenly established at the moment of insertion and continues at the same level for a considerable period. (2) The potential values of many insertions are coincident with each other in a range of 2～3 millivolts. (3) The observed value does not change when another electrode is inserted, if possible, into the same cell, or when the electrode is re-inserted at the point not apart farther than the cell-width. These are the criteria for judging the "membrane resting potential" (injury potential) and accordingly; (4) The main action potential (depolarization of the cell) is monophasic and is positively directed. (5) It is usual that an amplitude of action potential is greater than that of resting one (existence of "overshoot"). (6) The histological investigation is desirable for checking the position of the inserted electrode-tip or the cell-injury caused by the experimental procedure.

In Fig. 4 or Fig. 5, the electrophysiological technique or the method, except the exploring electrode, may be suitable for the purpose of intracellular potential recording. For example, the used amplifier had the "time-constant" more than 2

seconds, the grid-current of the head-tube was less than 10^{-14} amperes (checked by a special tube, UX-54A, Toshiba), the stray-capacity at the input was about 3.5 pico-farads and the D. C. amplifier was mainly used. The glass-capillary electrode which is used in the present experiment has a large tip-diameter and can not pick-up the intracellular potential, as stated above. However, the recorded action potentials in Fig. 4-B, 4-C, 5-A and 5-B are monophasic and positively directed. The sudden deflection towards negative potential level was observed at the moment of insertion and the level was maintained for about 10 minutes. Therefore, if ever the two findings among the above, (1) and (4), were recognized, the recording could not be interpreted as showing the intracellular potential. The finding (2) would be recognized in an extracellular recording where the injury were well demarcated by the syncytial character of the material. Therefore, the recognition of (3) or (5) may be most important for judging the intracellular action potential. It is regretable, however, that they were not ascertained in this study owing to the technical difficulties.

SUMMARY

The action potentials of cat's ureter, in situ, accompanying spontaneous peristalsis were recorded and an electrophysiological character of the ureteral muscle-cell was studied.

1) The features of the bipolar action potential were same as those reported by other investigators, and further studies on them were laid aside.

2) The monopolar action potential was three-phasic: i.e. the first positive-, second negative- and last positive waves were observed in the monopolar recordings. The first wave and the last one were interpreted as the "field-effect" caused by the coming and foregoing impulse, respectively. The second negative wave indicated the action potential of the muscle-cells around the exploring electrode.

3) The monophasic action potential could not be recorded through the suction electrode. Comparing it to the similar experiment on the frog's heart, this finding was thought to be caused by an insufficient suction or by the histological difference between the two preparations.

4) The tip of glass capillary electrode which was filled with Ringer's solution or with 3-molar KCl solution, was artificially broken and inserted into the ureter. The records obtained by using this capillary as an exploring electrode were monophasic, and the possibility to see the monophasic action potential by these procedure was discussed.

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