Generalia

Electrophysiological Studies on Gland Cells*

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Introduction

The physiology of nerves and muscles, as we know it today, could never have been developed without the extensive use of electrophysiological methods. In the physiology of gland cells, electrical methods have until recently only been used occassionally, a fact that explains the paucity of precise information on membrane events in secretory cells. Yet without such information we can hardly expect to get insight into the basic mechanisms of secretory processes. Fortunately, in recent years an increasing amount of papers describing electrophysiological aspects of gland cells have appeared. Because of the relatively small size of gland cells, as compared to nerve or muscle cells, a number of technical problems exist and have probably influenced the results of many reports. The present survey is not a review of gland cell electrophysiology, although a number of important results will be mentioned, but rather a critical examination of the methods currently in use.

Type of preparation

In the terminology of this review, cell electrophysiology means measurement of cell membrane potential and possibly cell membrane resistance. As discussed in a following section, this must be done with the help of glass micro-electrodes puncturing the cells. Several tissue preparations are at hand: a) in vivo, b) perfused glands, c) superfused segments or superfused slices, d) isolated or cultured cells. The choice of preparation depends, among many things, on the particular tissue under investigation and on the other parameters, besides the electrical ones, which are to be measured simultaneously (fluid secretion, enzyme output, hormone output, metabolism etc.).

a) The in vivo preparation will frequently provide the starting point, since it often appears to be the easiest to handle. This unfortunately frequently turns out not to be the case. One problem is movement of the tissue due to the blood circulation and the respiratory movements of the animal. Manipulations of the ionic composition of the extracellular fluid are very restricted, and the results obtained from studies where close arterial infusion of ions or chelating substances has been attempted are not always clear[1]. The main advantage of this preparation is that gland stimulation can be carried out using the natural nerve supply, and important information on physiological aspects of the type of innervation has been obtained[2,3]. It is also the duty of physiologists to measure electrical parameters under conditions as close to those in the intact living organism as possible, and especially in the salivary glands excellent results have been obtained[4].

b) This classical physiological preparation has repeatedly been the object of electrophysiological investigations, especially in the case of the salivary glands and the pancreas[5–8]. This preparation has many advantages, mainly because of the easy manipulation of the extracellular ionic composition, but the stability of the membrane potential recording is not very good. Close arterial injection of the appropriate physiological secretory stimulant (frequently acetylcholine (ACh)) often results in movements of vascular smooth muscles causing movements of the tissue. It is therefore only rarely possible to record the full time course of a stimulant-induced change in membrane potential, and in the perfused pancreas it is even difficult to record the initial effect of ACh or cholecystokinin-pancreozymin on the membrane potential[8]. Therefore, in spite of the fact that in this preparation a correlation between fluid secretion from an exocrine gland and changes in membrane potential is possible, there are severe limitations precluding a more detailled analysis of the electrical properties of the gland cell membrane.

c) The superfused slice preparation has frequently been employed and has properties similar to the superfused gland segment preparation. In the slice preparation, the micro-electrode impalement occurs through a cut surface, whereas in the segment preparation the natural surface of at least part of the gland is untouched and the impalement occurs through an undamaged

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surface. The main advantage of these types of preparations is that it allows very stable membrane potential measurement and also easy manipulation of the ionic environment. In the liver[9], the pancreatic islets[10], the exocrine pancreas[11,12], the adrenal cortex[13] and the salivary glands[14,15], this technique has been successfully employed. This type of preparation in some cases also allows simultaneous recording of membrane potential and output of the secretory product[16–18], (adrenal cortex, exocrine pancreas).

d) In some cases, where the slice or segment preparation has given less satisfactory results, e.g. the adrenal medulla, preparation of cultured cells[19] or isolated cells[20] has been useful. Generally measurement of membrane potentials in isolated cells is very difficult and often it has been impossible to record stable potentials of sufficient magnitude, so that measurement of initial transient higher potentials immediately following the impalement using high time resolution equipment has been employed[21]. In some fortunate cases, however, stable membrane potentials at a relatively high and therefore convincing level have been obtained[19,22]. In such cases isolated or cultured cells can be very useful, and they could become particularly useful in studies where the effect of artificial changes in membrane potential evoked by intracellular current injection on the stimulant-induced membrane potential change was under investigation, since in the isolated cells the influence of current flow from neighbouring electrically coupled cells does not exist.

Measurement of membrane potentials

In excitable tissues different techniques for measuring membrane potentials exist: 1. Capillary glass micro-electrodes which are inserted into the cell by puncturing the cell membrane[23–25]. 2. Larger micro-electrodes inserted axially into long cable-like structures with a large diameter (squid axons)[26,27]. 3. The sucrose gap technique used on tissues composed of long cells with smaller diameters (myelinated nerve, smooth muscles)[28]. Of these techniques only the first can be applied to the exocrine gland cells, since these are small cuboid structures. The fact that gland cells generally are much smaller than nerve and muscle cells, however, presents difficulties even for the micro-electrode technique and this has influenced many results unfavourably.

It has been realized since the beginning of the micro-electrode era that membrane potentials measured with this technique tend to give too low values[24], and this is true especially in the case of small cuboid cells[29]. All electrophysiologists face the problem of selection of data and therefore a discussion of the criteria used for such a selection is pertinent. If a sudden small advancement of the micro-electrode tip in a tissue causes a rapid change of the electrical potential of the electrode tip, which is stable within at least a few seconds, and the potential suddenly returns to the preadvancement level upon withdrawal of the electrode, this will often be considered a satisfactory cell impalement. However, as pointed out by FRÖMTER[29], there appears to be a positive correlation between the stability and the magnitude of the membrane potential. In my view, the key point is the stability of the recorded potential, and generally one should require that membrane potentials are stable in the range of minutes rather than seconds. Previously it was considered essential that the potential change upon micro-electrode impalement should be sharp. Unfortunately the term sharp is not well defined. Therefore it may be better to avoid this criterion since it is relative and mainly dependent on the speed of the pen recorder paper or the sweep of the oscilloscope beam. With standard equipment it is anyway impossible to know what happens in the first milliseconds following the micro-electrode impalement. The essential question is whether the potential following the impalement, observed on a minute time scale, is declining, remaining, or increasing and then stabilizing. The two latter patterns will represent satisfactory impalements. In Figure 1 examples of different types of impalements are given.

There has been some discussion over the meaning of the absolute value for the membrane potential obtained from micro-electrode work. It has been correctly pointed out that such a measurement from the standpoint of a physicist is meaningless[30]. However,

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in those cases where the membrane potential can be related to specific ionic transmembrane gradients, i.e. where it can be shown that the membrane potential depends on the extracellular concentration of K^+, the potential value gains physiological meaning. In several gland cell types, it has been possible to make such a correlation. Figure 2 shows the linear relation between the parotid acinar cell membrane potential and the logarithm of the extracellular K^+ concentration.

Since glands are non-stationary organs which are activated by specific stimulants, transmitters or hormones, the most important aspect of membrane potential measurement is to allow a detailed analysis of the membrane action of secretory stimulants. For this purpose, however, potential measurements alone are not enough and it becomes necessary to include measurement of membrane resistance.

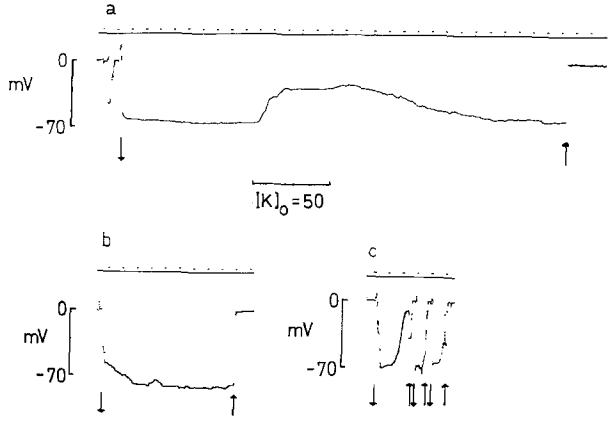

Fig. 1. Membrane potential measurement in mouse parotid acinar cells. Superfused segment preparation. ↓ indicates cell impalement, ↑ withdrawal of micro-electrode. In a the effect of replacing the standard Krebs-Henseleit solution by a solution containing 50 meq/l of K^+ was tested. a) and b) represent satisfactory cell impalements whereas in c) examples are shown of unsatisfactory unstable membrane potential measurements. Distance between time markings = 1 min.

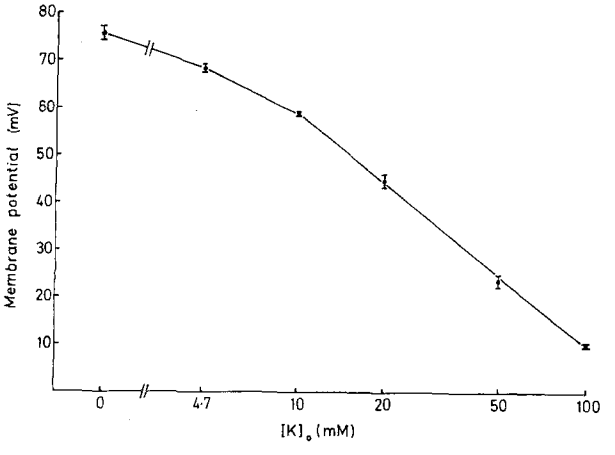

Fig. 2. Relationship between resting acinar cell membrane potential in the mouse parotid and the extracellular potassium concentration (from PEDERSEN and PETERSEN[15]).

Measurement of membrane resistance

Since we can only measure membrane potentials in exocrine gland cells using the micro-electrode technique, it is clear that we also have to use this technique for measurement of membrane resistance. However, several possibilities still exist. Theoretically the best solution would be to insert two micro-electrodes into the same cell, one for recording the membrane potential, the other for passing current. The quotient membrane potential/current strength would then, according to Ohm's law equal the membrane resistance. Unfortunately this technique which is used with much success on striated muscle cells[31,32], cannot be applied in the case of the exocrine glands because of the small cell size.

Exocrine gland cells are electrically coupled[9,20], due to the presence of low resistance junctional membranes[33]. It is therefore possible to insert 2 electrodes in 2 different, not necessarily adjacent, cells and record a change in potential from one cell during injection of current into another[9]. In this case absolute values for input resistance cannot be arrived at without knowing the cable properties of the tissue. However, the method allows measurement of changes in membrane resistance evoked by stimulation. Thus HAYLETT and JENKINSON[9] were able to demonstrate that noradrenaline evoked a decrease in liver cell membrane resistance using this method.

A third possibility is to insert a double-barrelled micro-electrode into a cell and use one barrel for recording and the other for passing current[34]. This technique is difficult mainly due to unpredictable changes in coupling resistance between the two barrels. It has been used with partial success in studies on cat sublingual gland cells[35].

The technique which has given the best results is to use only one single micro-electrode and pass current through this recording electrode. There will be two resistances, in series, to the injected current: The electrode tip resistance and the cell input resistance. Hence the voltage drop caused by current injection will in this case occur partly at the tip of the micro-electrode and partly at the level of the cell membrane. We therefore need a circuit to compensate for the voltage drop across the electrode tip resistance. Several ingenious methods developed for this purpose have been described. The first good circuit devised was the bridge balance system by ARAKI and OTANI[36], which has

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since been used repeatedly also in gland cells[4,37]. In this case the micro-electrode was placed together with the cell membrane in one arm of a Wheatstone bridge.

A somewhat different method, in which the electrometer amplifier is not connected to an external bridge circuitry, has been devised by FEIN[38]. This electrometer amplifier has two outstanding features. 1. It allows current injection through a recording micro-electrode without in any way degrading the performance of the electrometer amplifier system. 2. The electrode tip resistance compensation is achieved by employing a differential amplifier of an oscilloscope as a null detector between the output from the electrometer amplifier and the output from a balance potentiometer. In practical micro-electrode work, this system has been extremely useful and with the help of this circuit many features of the pancreatic acinar cell electrophysiology have recently been worked out[39–41]. Figure 3, shows a block diagram of the circuit used in these investigations[40,41]. In Figure 4 is shown a typical recording of membrane potential, resistance and time constant from a pancreatic acinar cell using this technique. As already mentioned, it is the general notion that gland cells are electrically coupled, i.e. the specific resistance of the junctional membranes is much lower than that of the surface cell membranes. This means that, from the input resistance of a gland cell, we cannot calculate the specific membrane resistance by additionally simply estimating the cell surface area. In a tubular structure one could measure the voltage spread into adjacent cells caused by intracellular current injection. However, such measurements can only yield qualitative values for the specific membrane resistance, since an exact solution for the voltage spread around a current point source is not possible in a tubular structure with a conducting core[29].

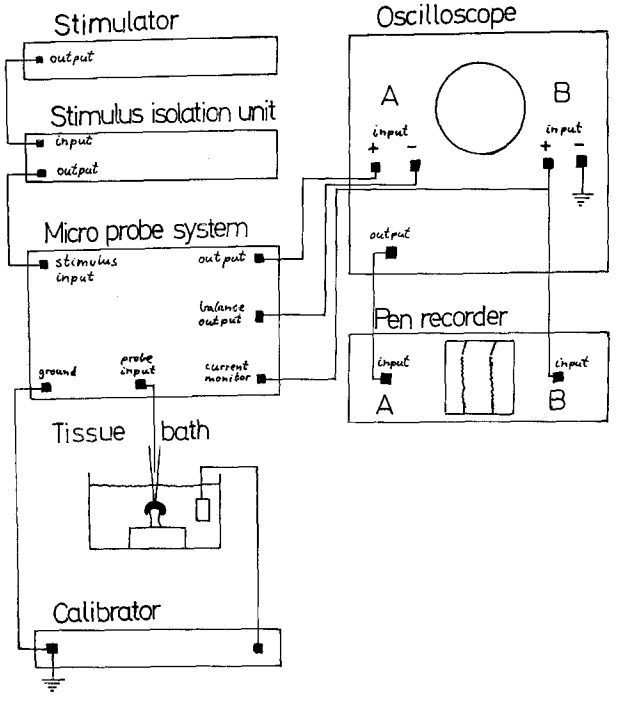

Fig. 3. Block diagram of circuit used for measurement of membrane potential and resistance employing a single micro-electrode. For further explanation see text.

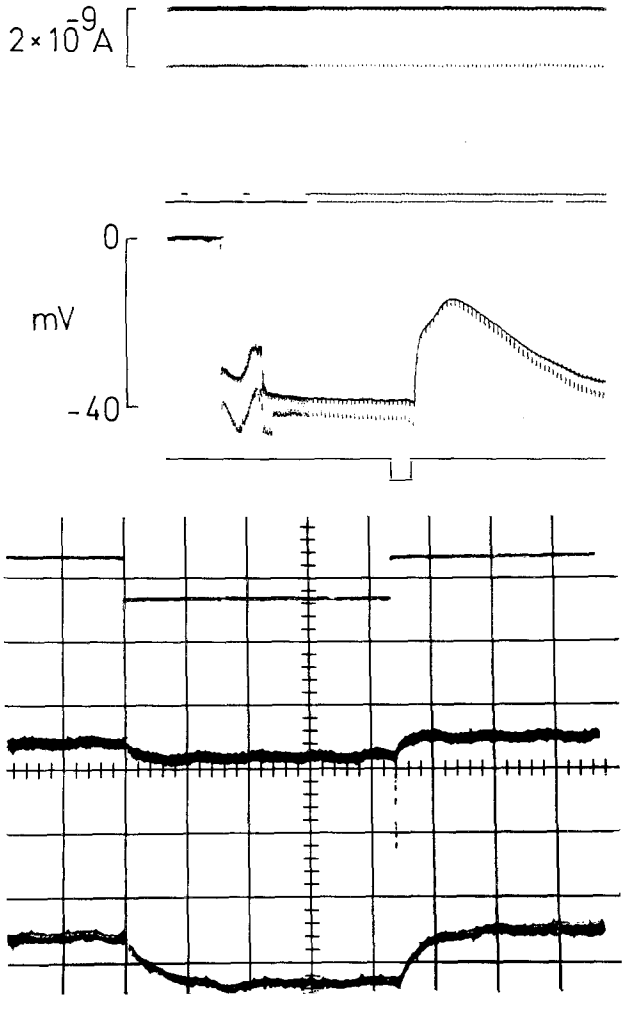

Fig. 4. Measurement of membrane potential and resistance in mouse pancreatic acinar cell from superfused gland segment preparation using the method outlined in Figure 3. Upper part: pen recordings. From top: current monitor trace, time marker trace (large pulses occurring every minute), membrane potential trace and event marker trace (pulse indicating injection of ACh into the tissue bath). Lower part: Oscilloscope screen photograph. Upper trace represents the current signal whereas the two lower tracings represent the current-induced membrane potential change in the resting state (bottom) and during the action of ACh (middle trace). Note reduction in time constant evoked by ACh. Calibration: Horizontal 20 msec/division, vertical 3×10^{-9} A or 5 mV / division (from NISHIYAMA and PETERSEN, unpublished observations).

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Although it is, of course, always desirable to express results in a quantitative way, the essential and probably most useful feature of electrophysiological investigations on gland cells is the measurement of *changes* in input resistance after application of the appropriate physiological stimulant, and especially the ionic dependence of this response. This provides us with information about the ionic species moving across the gland cell membrane during the action of hormone or transmitter. It could be argued that, from the measurement of input resistance alone, it is not possible to distinguish between changes in surface cell membrane resistance and changes in junctional membrane resistance. However, if time constants are also measured (Figure 4), qualitative statements can be made. The time constant is equal to the product of resistance (R) and capacitance (C). A decrease in surface cell membrane resistance means solely a decrease in R and therefore a reduction in time constant. A decrease in junctional membrane resistance increases C by making a larger surface membrane area available for the injected current to traverse. This effect would thus cause a decrease in input resistance but only a small reduction or perhaps an increase in time constant. It is therefore obviously very important to measure membrane time constants to carry out this type of analysis. There is one technical problem. If the microelectrode tip resistance is too high (more than about 50 MΩ), it is usually difficult to separate the electrode time constant from the membrane time constant. It is therefore necessary in this kind of studies to use micro-electrodes with tip resistances below 40 MΩ. In cells with very small dimensions, such as the β cells of the pancreatic islets, this may cause serious problems.

Conclusion

In order to elucidate the mechanism of action of secretory stimulants on gland cell membranes, electrophysiological methods are extremely useful. At present micro-electrode studies are best performed in superfused gland segments because of the very good stability of this system. Using recently developed equipment, it is possible with the help of only 1 microelectrode also to measure membrane resistance and membrane time constant, thus obtaining information about relative ionic permeabilities.

Zusammenfassung. Betonung elektrophysiologischer Methodik zum Nachweis der Einwirkung physiologischer Stimulatormoleküle auf die Zellmembran. Vorteil von Schnitt- oder Segmentpräparaten bei grösster Stabilität für Membranpotentialmessungen mit Mikroelektroden. Entwicklung neuer Methoden zur Erlangung von Informationen über relative Ionenpermeabilität mittels einer Mikroelektrode bei alleiniger Messung von Membranwiderstand und Zeitkonstante.

SPECIALIA

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Gliovictin, ein neuer Metabolit von *Helminthosporium victoriae*

Im Verlaufe von Untersuchungen über Victoxinin und verwandte Sesquiterpene von *Helminthosporium victoriae*[1] sind wir auf einen Metaboliten gestossen, der eindeutig einer andern Stoffklasse zuzuordnen ist. Wir berichten im folgenden über seine Struktur.

Die neue Verbindung, Smp. 134°, $[\alpha]_D^{25} = -65°$ ($CHCl_3$), der wir den Namen Gliovictin geben möchten, konnte aus dem Neutralteil des Kulturfiltrates durch Extraktion mit Aether und anschliessende Chromatographie an Silicagel in Mengen von ca. 40 mg/l isoliert werden. Zusammen mit den Resultaten der massenspektrometrischen Untersuchungen ergab die Verbrennungsanalyse die Zusammensetzung $C_{16}H_{22}N_2O_3S_2$ (ber: C 54.23, H 6.26, N 7.91, S 18.10%; gef: C 54.12, H 6.14, N 8.09, S 18.10%). Gliovictin weist im IR-Spektrum ($CHCl_3$) Banden bei 1650 cm^{-1} für Amidgruppen und bei 3570 und 3450 cm^{-1} für eine teilweise assoziierte Hydroxylgruppe auf. Im IR-Spektrum (CCl_4) des leicht herstellbaren *O*-Acetyl-Derivats, Smp. 131°, $[\alpha]_D^{25} = -77°$ ($CHCl_3$), ist neben der neu hinzugetretenen Carbonylbande bei 1750 cm^{-1} keine Amin- bzw. Hydroxylabsorption zu erkennen. Im NMR-Spektrum (100 MHz, $CDCl_3$) des Gliovictins treten Signale für zwei CH_3S-Gruppen (δ = 2.18 und 2.35), für zwei CH_3N-Gruppen (δ = 3.08 und 3.35) und für fünf aromatische Protonen (δ = 7.2, m) auf. Ein ABX-Signal (δ_A = 3.90, δ_B = 3.18, δ_X = 1.30; J_{AB} = 12, J_{AX} = 7.5, J_{BX} = 7 Hz), welches bei der Zugabe von D_2O zu einem AB-Signal (δ_A = 3.88, δ_B = 3.17; J_{AB} = 12 Hz) vereinfacht wird, weist auf die Anwesenheit einer tertiär gebundenen Hydroxymethylgruppe hin. Beim *O*-Acetyl-Derivat von Gliovictin sind die entsprechenden Signale nach δ = 4.43 bzw. 4.29, J = 11 Hz, verschoben. Schliesslich ist die Lage eines AB-Systems bei δ = 3.17 und 3.77 mit J_{AB} = 14 Hz kongruent mit dem Vorhandensein einer benzylischen Methylengruppe ohne vicinal benachbarte H-Atome. Das Massenspektrum von Gliovictin bestätigt die An-

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