Secretory Potentials in Rat Submaxillary Gland* (33519)

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Stimulation of the secretory nerves to the submaxillary or sublingual gland in cat has been shown by Lundberg (1) to produce consistent changes in transmembrane potential of the parenchymal cells. These changes from the resting values constitute the secretory potentials and probably reflect ionic shifts related to cellular secretory processes (1). The form of the secretory potential (i.e., hyperpolarization or depolarization) in cat salivary gland evidently depends upon the type of cell which is stimulated. Furthermore, the magnitude of the normal resting potential also depends upon cell type. Thus, in cat submaxillary or sublingual gland Lundberg (1) found that cells with resting potentials near 20 mV consistently responded to parasympathetic or sympathetic stimulation by hyperpolarization (type I response), and that these cells were visually identifiable as acinar. In submaxillary, but not in sublingual gland, two other types of response to stimulation were found, but these were less prevalent than type I. Type II responses are characterized by hyperpolarization during parasympathetic stimulation and depolarization during sympathetic stimulation. Responses of this type were elicted from cells with resting membrane potentials averaging 32 mV; the histological identity of the cells is uncertain. Type III responses show depolarization during stimulation of either autonomic branch and are derived from cells with resting potentials normally near 80 mV in magnitude. These cells are probably ductal (1). Lundberg's observations on secretory potentials and the histological identity of the cells with low resting potentials have been amply confirmed in cat (2, 3) and in dog (4). However, the ionic basis of the potentials is still not well understood.

Although secretory potentials have been most extensively studied in acinar cells of cat and dog, the electrolyte composition of acinar secretion has been directly determined only in rat salivary glands (5–7). Hence it is of interest that Ichioka *et al.* (8) have reported that in rat submaxillary gland secretory potentials elicited by reflex stimulation are inconstant in form. Thus depolarization or hyperpolarization, or both in sequence, could be observed when low-potential acinar cells were stimulated by application of sapid substances to the tongue. Although no explanation for this inconsistency was provided (8), there is the possibility that acinar cells of rat submaxillary gland show type II responses and that stimulation by way of gustatory receptors activates both parasympathetic and sympathetic pathways. Because of this possibility, an investigation of secretory potentials was undertaken using electrical stimulation of the separate autonomic branches to rat submaxillary glands. In this investigation only secretory potentials from acinar cells, i.e., cells with resting potentials less than 35 mV (9) were studied. It was found that the secretory potentials were generally small in magnitude and inconstant in form, even though elicited by direct stimulation of the separate parasympathetic or sympathetic innervation.

Methods. Long-Evans adult rats were surgically prepared for microelectrode recording after administration of sodium pentobarbital (50 mg/kg, i.p.) for anesthesia. The main excretory duct of a submaxillary gland was cannulated at its oral end, using fine polyethylene tubing. The submaxillary gland was exposed along its ventral surface and cleared of capsular connective tissue. The sublingual gland was removed. The main submaxillary excretory duct was gently separated from sur-

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rounding connective tissue and muscle inferiorly from the hilus of the gland to a point close to the level at which the duct is crossed by the lingual nerve. Bipolar platinum or silver electrodes, insulated to within a few millimeters of their tips, were placed high on the main excretory duct. Earlier work (10) showed that stimulation of chorda tympani fibers along the duct evokes saliva of parasympathetic character. For stimulation of the sympathetic innervation, electrodes were placed around the common carotid artery or on the superior cervical ganglion. The trachea was cannulated to facilitate management of respiratory complications. A Grass stimulator with stimulus isolation unit was used to provide repetitive shocks of 10–20 cps frequency at 2-8 V (5 msec duration), which have previously been shown to provide near maximal to maximal secretory responses (10). Micropipettes with approximately 0.5-μ tip diameters were prepared from Kimax tubing (0.7–1 mm outside diameter), using a solenoid-activated micropipette puller, and were filled with 3 *M* KCl. Tip potentials of acceptable microelectrodes were usually less than 3 mV, and resistances were between 3 and 30 megohms. The microelectrode assembly and recording system were similar to those used previously (9, 11). In outline, KCl-filled micropipettes were connected by way of an agar bridge (2% agar in 0.15 *M* NaCl) to a chlorided silver wire which led to the high-impedance input of a negative-capacity electrometer amplifier (Medistor). A second agar bridge with its Ag–AgCl wire served as indifferent electrode and led from the surface of the gland to the indifferent lead of the amplifier. Brinkmann micromanipulators were used to control the position of the electrodes. The output of the amplifier was led to an oscillographic recor-

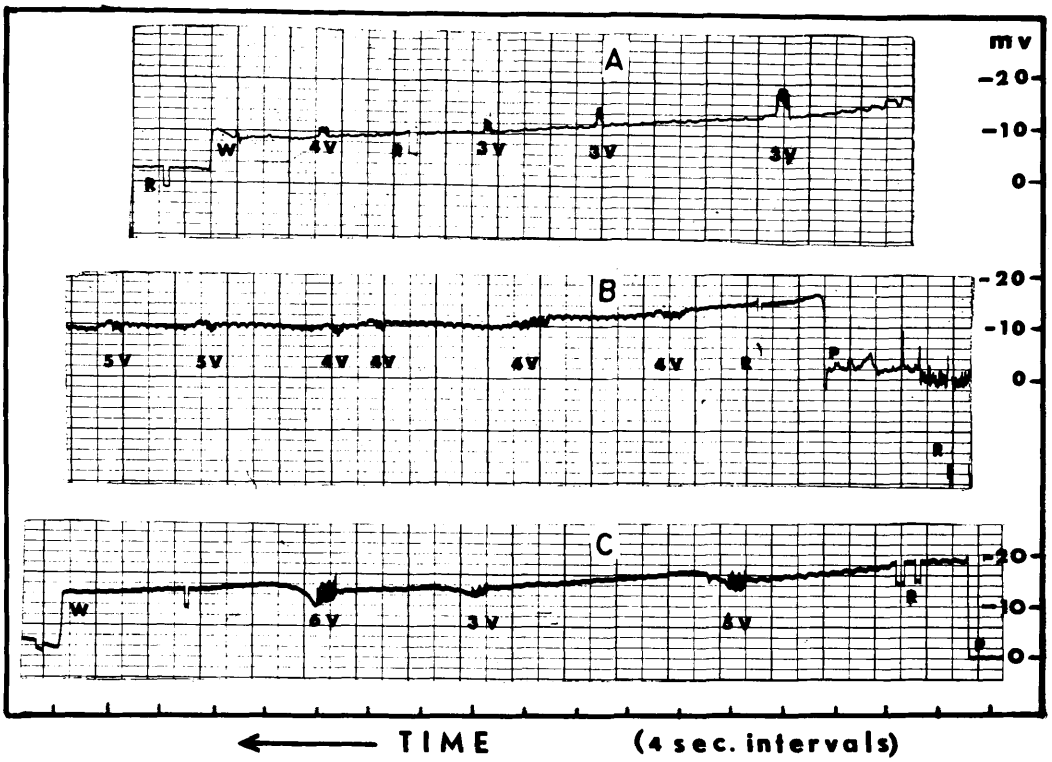

FIG. 1. Representative recordings of submaxillary secretory potentials evoked from acinar cells by electrical stimulation of the parasympathetic innervation. The period of stimulation is indicated by the shock artifact; stimulus voltage, at 10–20 cps, is indicated at each period. Symbols: P = penetration of cell by microelectrode; R = microelectrode resistance, recorded as megohms on the ordinate scale; W = withdrawal of microelectrode from cell.

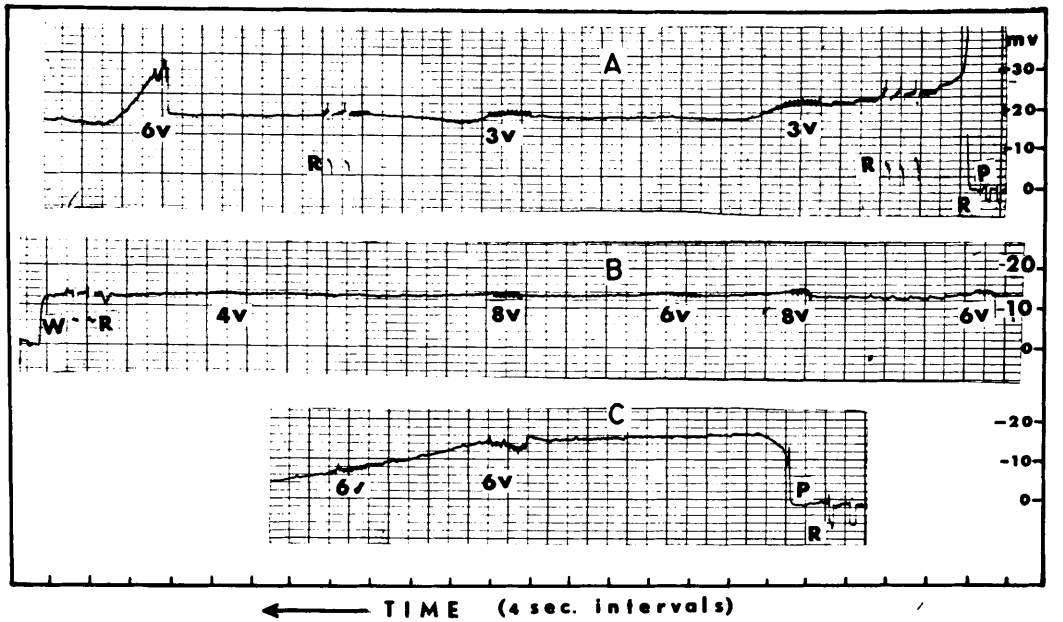

FIG. 2. Representative recordings of submaxillary secretory potentials evoked from acinar cells by electrical stimulation of the cervical sympathetic trunk. The period of stimulation is indicated by the shock artifact; stimulus voltage, at 20 cps, is indicated at each period; symbols are explained in Fig. 1.

der (Sanborn 7701A, with 8802A preamplifier). Electrode resistance was monitored by use of a current-sending circuit which is a component of the amplifier.

Results and Discussion. Oscillograph records showing representative secretory potentials from acinar cells (mean resting potential, 22 mV) are shown in Figs. 1 and 2. Records in Fig. 1 were obtained in response to stimulation of the parasympathetic innervation and indicate the variation which was observed in the form and magnitude of the response. In some instances, the secretory potential consisted of a distinct hyperpolarization (Fig. 1A) which is reminiscent of the typical response reported for acinar cells of cat (1, 2) and dog (4) salivary glands. In other cases depolarization to variable extent comprised the major change during stimulation (Fig. 1B and C). In many instances, hyperpolarization appeared as a delayed response after the depolarization which occurred during the period of stimulation (Fig. 1C). In other cases, there was no change from the resting value during stimulation, but in some of these instances a delayed hyperpolarization occurred. Data summarizing all of the findings are presented in Table I. Hyperpolarization accounted for only 20% of responses. While no change in potential occurred during stimulation in 29% of cases, with repeated intervals of stimulation during the same impalement some change could always be elicited. The form of the response during multiple periods of stimulation of the same cell frequently did not remain constant as shown by the overlap in percent of cells showing each of the three responses tabulated in Table I. In all cases, the magnitude of the secretory potential was notably small.

When sympathetics were stimulated, the response was also highly variable. Representative recordings are shown in Fig. 2. Hyperpolarization in response to stimulation can be seen in Fig. 2A and B; in Fig. 2C depolarization occurs (first response). Data from 137 periods of sympathetic stimulation are summarized in Table I. Except for a possible small difference in magnitude, some difference in the frequency distribution of the types of response, and the absence of observed hyperpolarization as a delayed re-

sponse when none had occurred during stimulation, the pattern of secretory potentials was similar with sympathetic or parasympathetic stimulation (Table I).

TABLE I. Changes in Submaxillary Transmembrane Potential[a] during Electrical Stimulation of the Automatic Innervation.

	Hyperpolarization	Depolarization	No change
Parasympath. stim. (144 stimulations, 45 cells in 7 rats)			
(mV) mean	2	2	0
range	(1–8)	(1–21)	
% of responses	20	49[b]	29[c]
% of cells[d]	29	69	51
Sympath. stim. (137 stimulations, 54 cells in 4 rats)			
(mV) mean	4	6	0
range	(1–14)	(1–20)	
% of responses	35	39[e]	25
% of cells[d]	46	67	33

[a] Resting potential, 22 mV (av).

[b] Hyperpolarization (5 mV, av) occurred as a delayed response (after cessation of stimulation) in 59% of these cases of depolarization.

[c] Delayed hyperpolarization (9 mV, av) appeared in 34% of these cases.

[d] Percentage of impaled cells with at least one response in an indicated category. Of the cells which showed no change during a period of stimulation, none failed to respond at all when multiple periods of stimulation were employed.

[e] Delayed hyperpolarization (6 mV, av) appeared in 15% of these cases.

With regard to the variability of the form of the secretory potential in rat submaxillary cells, the results generally confirm the earlier findings of Ichioka *et al.* (8). However, those earlier findings were obtained by a method of stimulation (reflex stimulation, by way of gustatory receptors) which left open the possibility that both autonomic branches could be acting during a single period of stimulation. If Lundberg's type II response were prominent in rat submaxillary gland, secretory potentials of mixed form could have resulted from such stimulation. The results from the present study in which the secretory nerves were separately stimulated, show that inconstant form is characteristic of the secretory potential in rat submaxillary cells. The reasons for this inconstancy are uncertain. In preliminary work, on 2 rats (43 stimulations during 24 impalements), administration of atropine (1 mg/kg of body wt., i.p.) prior to parasympathetic stimulation was observed to block the appearance of any secretory potentials in submaxillary cells when overt secretion could not be obtained. Since atropine does not block the changes in blood flow which occur during parasympathetic stimulation (12), it appears unlikely that the secretory potentials are artifacts due to vascular changes in the gland. Instead, there is a possible pattern among exocrine glands with regard to characteristics of the acinar secretory potential. In one group, exemplified by submaxillary and sublingual of cat, hyperpolarization to appreciable degree is the prevalent response. In cat parotid and dog submaxillary glands, hyperpolarization is still characteristic, but the magnitude of the response is less (3, 4). In rat submaxillary, hyperpolarization is small in magnitude and inconstant in occurrence. Depolarization is frequently observed instead. In lacrimal gland of cat, depolarization followed by hyperpolarization is evidently the predominant response (13). It seems possible that the secretory potential is attributable to more than one ionic shift (1, 4, 13, 14) and that the form of the potential depends upon an algebraic balance of these ionic movements at any point in time.

Summary. Changes in membrane potential in relation to stimulation (secretory potentials) were investigated in cells of rat submaxillary gland. In acinar cells, stimulation of the parasympathetic or sympathetic innervation evoked secretory potentials which were small in magnitude and inconstant in form. Either hyperpolarization or depolarization occurrred when each autonomic branch was separately stimulated. It is suggested that more than one ionic shift occurs across the membrane during stimulation and that the form of the potentials depends upon an algebraic balance of ion movements at any time.

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Experimental Infection of Rhesus with Simian Virus 40 (SV40)* (33520)

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Although simian virus 40 (SV40) is a common infection of rhesus (1) and a frequent contaminant of rhesus kidney cultures (2), little is known of the course of SV40 infection in rhesus and of the manner in which virus disseminates from an infected animals.

Meyer *et al.* (3) and Ashkenazi and Melnick (4) reported on some aspects of SV40 infection in African green monkeys. We studied experimental infection of juvenile nonimmune rhesus with SV40 by three routes of inoculation, and reported earlier the viremia and T antibody response in these animals (5). In the present communication, the remaining features of this infection and attempts to infect rhesus *in utero* are described.

Materials and Methods. All rhesus in this study were obtained from the free-ranging groups on Cayo Santiago, an islet off the east coast of Puerto Rico. This island is a part of the facilities of the Laboratory of Perinatal Physiology. These rhesus groups are free of active SV40 infection (6).

Strain A2895 of SV40, isolated originally from a hamster tumor produced by inoculation of rhesus kidney extracts (7), was obtained from Dr. B. Eddy. Prior to use in this study the virus had undergone at various times one additional passage in hamster tumor, one passage in HeLa cells, and 10 passages in *Cercopithecus* kidney cells, either primary cultures or continuous cell line BS-C-1.

Sixteen juvenile rhesus, 1–2 years old, were infected, six each by intranasal (i.n.) and subcutaneous (s.c.) routes and four by intragastric (i.g.) route. The virus dose for each animal in i.n. and s.c. groups was $10^{7.3}$ TCD_{50} contained in 0.4 ml of a $10^{-1.0}$ dilution of the virus. For i.g. inoculation, 1 ml of undiluted virus containing $10^{8.7}$ TCD_{50} was introduced into the stomach of each animal

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