*J. Physiol.* (1974), **236**, *pp.* 723–731
*With 7 text-figures*
*Printed in Great Britain*



# CONDUCTANCE CHANGES ASSOCIATED WITH THE SECRETORY POTENTIAL IN THE COCKROACH SALIVARY GLAND

BY B. L. GINSBORG, C. R. HOUSE* AND E. M. SILINSKY

*From the Department of Pharmacology, University Medical School, 1 George Square, Edinburgh EH8 9JZ and the *Department of Physiology, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh EH9 1QH*

(*Received 10 September 1973*)

## SUMMARY

1. Conductance changes in the acini of the cockroach salivary gland have been examined during nerve stimulation by means of two intracellular electrodes placed in the same acinus, the first electrode being used for recording membrane potential and the second for current injection.

2. The transient hyperpolarization (secretory potential) in the acinus evoked by nerve stimuli is accompanied by a rise in membrane conductance. The conductance, however, remains high for a longer period than that of the response.

3. Applying the analysis of Trautwein & Dudel (1958) to the secretory potentials recorded in the acinus (assumed to behave electrically like a single cell) gives estimates of the 'transmitter equilibrium potential'. The values indicate that the neurotransmitter increases the membrane potassium conductance.

4. The hyperpolarization of the acinus evoked by $10^{-6}$ M dopamine in the bathing fluid is also associated with an increase in membrane potassium conductance.

## INTRODUCTION

It has been shown recently that stimulation of the nerve supply that evokes secretion from the salivary gland of the cockroach (Whitehead, 1971) causes a hyperpolarization of the acini which may be recorded with an intracellular electrode (House, 1973; Bland, House, Ginsborg & Laszlo, 1973). This electrical response is presumably homologous with the secretory potentials that can be recorded from mammalian preparations and since their origin remains somewhat puzzling it was of interest to examine the response in the cockroach. In the mammal the amplitude of the response

depends on the external ion concentrations as if it were generated by an increase in potassium permeability (Imai, 1965; Yoshimura & Imai, 1967; Petersen, 1970). One way of testing whether a similar mechanism operates in the cockroach gland is to examine the effect of changing the membrane potential of the gland cells during nerve stimulation; in particular, it should be possible to 'invert' the secretory potential by a sufficiently large hyperpolarization. This was the main object of the present experiments.

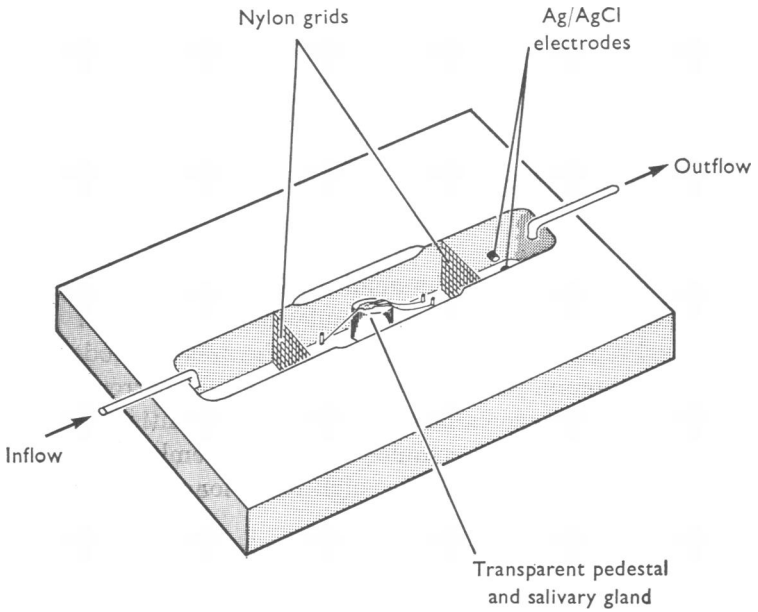


Fig. 1. Diagram of experimental chamber.

## METHODS

Salivary glands were dissected from adult cockroaches, *Nauphoeta cinerea* Olivier, maintained in culture as described previously (House, 1973). The preparation was mounted in the chamber shown in Fig. 1 and stretched over the transparent pedestal by means of three silk ligatures. Two ligatures were attached to the salivary reservoirs associated with the paired glands and the third tied to connective tissue joining the glands anteriorly. The main ducts of the salivary glands and the reservoirs were drawn into a suction electrode used for stimulating the salivary nerves which are embedded in the ducts of the reservoirs and glands (Whitehead, 1971).

The preparation was bathed routinely at room temperature in a fluid containing 160 mM-NaCl, 1 mM-KCl, 5 mM-$CaCl_2$, 1 mM-$NaHCO_3$ and 1 mM-$NaH_2PO_4$. In some experiments the potassium concentration was changed to 5 mM or 10 mM by addition of KCl. The bathing solution was pumped through the chamber at a rate of about 2 ml. $min^{-1}$ by a Watson-Marlow H. R. Flow Inducer (MHRE 200) except when dopamine (Sigma Chemical Co.) was applied to the preparation at a maximal rate of

about 20 ml. $min^{-1}$. Non-turbulent perfusion was achieved by placing Nylon grids between the inflow and outflow tubes (Fig. 1).

Conductance changes in an acinus were monitored by injecting current pulses through an intracellular electrode and recording the electrotonic potentials with another intracellular electrode placed in the same acinus. The electrodes were filled with 3 M-KCl by the method described by Thomas (1972) and had a resistance of 10–20 MΩ; the bath electrodes were solid Ag/AgCl pellets as described by Beranek, Martin & Wickelgren (1970). The current monitor circuit was identical to that of Gage & Eisenberg (1969) except that the operational amplifier was an E-78B differential amplifier (Computing Techniques Ltd). The current electrode could be connected alternatively to the input of a second recording circuit. Thus, it was possible to confirm that satisfactory electrical coupling between different cells in an acinus was achieved only when both electrodes were genuinely intracellular and hence capable of recording similar secretory potentials evoked by nerve stimulation (see Fig. 2).

Short trains (25 at 100 Hz) of stimulating pulses (0·5 msec, 10–60 V) from a square pulse stimulator were delivered to the suction electrode. The interval between such trains was always larger than 2 min so that maximal and reproducible responses could be obtained (see House, 1973). The membrane potential of the gland cells was recorded with a high impedance preamplifier (WP Instruments) connected to a Tektronix 502 oscilloscope. Permanent experimental records were obtained with a Brush 220 recorder.

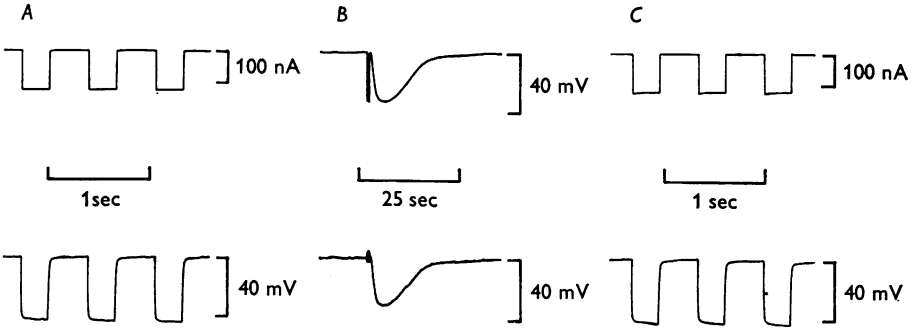


Fig. 2. Electrical coupling between cells. *A*: upper trace, current pulses; lower trace, electrotonic potentials. *B*: responses simultaneously recorded from two intracellular electrodes. *C*: as in *A* but after recovery from responses.

## RESULTS

### *Electrical coupling between cells in the same acinus*

It does not at present seem feasible to insert two micro-electrodes into the same cell, but the possibility of good electrical coupling in epithelia (see Loewenstein & Kanno, 1964; Frömter, 1972) suggested that significant results might be obtained with the electrodes in different cells of the same acinus. Fig. 2 shows the results of such an experiment. In *A* the membrane potential was recorded with the first electrode (lower trace) and current pulses were passed through the second electrode (upper trace). Entry into

the acinus was signalled by the appearance of the voltage pulses on the lower trace. The second electrode was then disconnected from the current supply and reconnected to a second recording circuit (see Methods). When the nerve trunk was stimulated almost identical responses were recorded on the two channels (2*B*). Results of a similar nature were obtained with many other double impalements in the same acinus but it should be emphasized that the responses were often *not* identical. Since larger discrepancies seemed associated with smaller voltage changes for a given current, it is at least possible that they were due to one of the two electrodes being improperly inserted. At any rate, it seemed worth pursuing the experiments in the hope that the approximation that the whole interior of the acinus was at the same electrical potential, so that the acinus might therefore be treated as a single spherical cell, would not be too misleading.

From the experiments in which the second electrode was used both for recording and current passing, it appeared that where the 'resistance' was less than 0·2 MΩ, the discrepancies between the responses were large: only impalements in which this value was exceeded were regarded as successful. Large 'apparent' resistances were sometimes obtained, however, when neither electrode was inside a cell. The voltage pulse on these occasions had the same rapid time course as that of the current pulse. When the two electrodes were in distant acini, no coupling potential was observed.

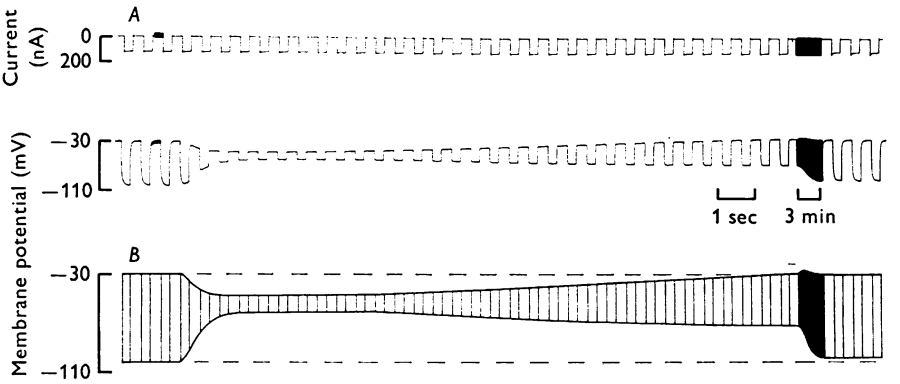


Fig. 3. Conductance change during response to nerve stimulation. *A*: upper trace, current pulses; lower trace, electrotonic potentials. Period of stimulation indicated by thickening of traces due to stimulus artifacts. $[K]_o$ = 10 mM. *B*: upper and lower envelopes of voltage trace in *A* (see text).

*Effect of nerve stimulation on the acinar resistance*

In Fig. 3*A* the effect of the current pulses is superimposed upon the secretory potential evoked by nerve stimulation. The results strongly suggest that the electrical response can be inverted. Thus, in 3*B* the upper envelope of the voltage trace represents the response at the resting potential; the lower envelope represents the response that would have been

observed had a *steady* current equal to the pulsed current been passed between inside and outside of the acinus.

Attention will be devoted to the 'early' part of the secretory potential. It can be seen that the conductance remains high even though the membrane potential recovers. It is possible that this is related to the after depolarization which is a prominent feature of the response in some cells (House, 1973) and it must certainly contribute to the length of the interval required between bursts of stimuli needed to obtain constant responses.

It therefore seems reasonable to try to interpret the secretory potential as if it were a conventional synaptic potential generated by an additional ion pathway, as in Fig. 4 (see e.g. Katz, 1966, p. 68).

### *Analysis of records*

As pointed out by Trautwein & Dudel (1958), values for the conductance, $g$, and the 'transmitter equilibrium potential', $e$, at the peak of the response may be derived from a single record of the kind shown in Fig. 3. For the convenience of the reader relations equivalent to those of Trautwein and Dudel are derived here as follows (see Fig. 4). Evidently for a current pulse $I$ giving electrotonic potentials $P$ and $p$ at rest and during the response respectively we have

$$P = \frac{I}{G} \quad \text{and} \quad p = \frac{I}{G+g},$$

where $G$ is the resting conductance in the circuit in Fig. 4*A*. For a resting potential $E$ and a response $v$ (see 4*B*) we find

$$g = \left[\frac{P-p}{p}\right] G \tag{1}$$

and

$$e = \left[\frac{P}{P-p}\right] v + E. \tag{2}$$

These relationships could not be valid unless, as is fortunately the case, the resting conductance were independent of the current and hence of the membrane potential. Applying (1) and (2) to the results shown in Fig. 3 gives

$$g/G = 5{\cdot}1 \quad \text{and} \quad e = -51 \text{ mV}$$

(the value for $e$ in this experiment was less negative than was typical).

Fig. 5 shows an experiment in which the conductance before and during two responses ($A$ and $B$) was investigated in the same acinus without withdrawal of the electrodes between the responses. In $B$ the current pulse was twice that in $A$ but the resting conductance remained the same ($3{\cdot}3\ \text{M}\Omega^{-1}$). The response in $B$ was evidently inverted at the membrane potential corresponding to the current-induced hyperpolarization; in $A$ the corresponding response was reduced, but not reversed, with the smaller degree

of hyperpolarization. However, almost identical values for the transmitter equilibrium potential were calculated from the records at the peak of the responses ($e = -68$ mV in $A$ and $-69$ mV in $B$) and also for the ratios $g/G$ (2·1 in both cases).

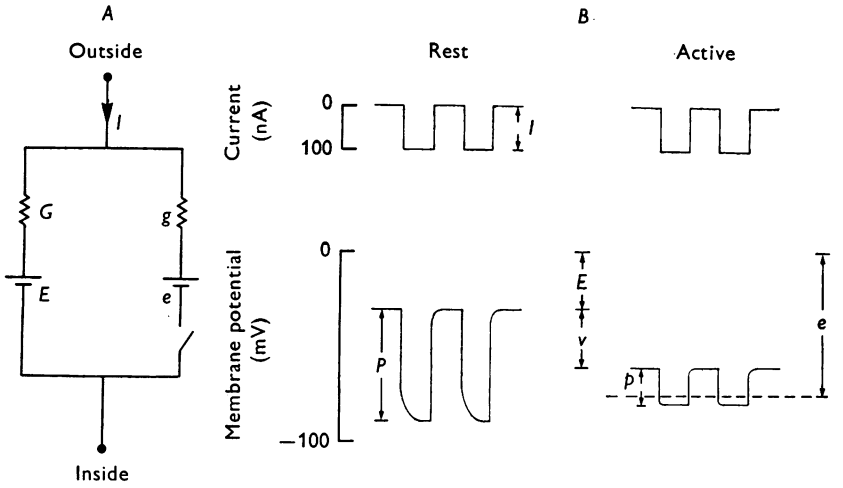


Fig. 4. Analysis of records. $A$: equivalent circuit. $B$: diagram to illustrate symbols used in text.

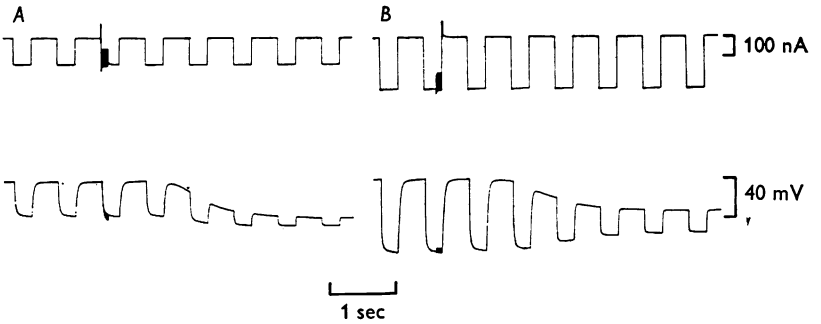


Fig. 5. Effect of current strength. $A$ and $B$: upper traces, current pulses; lower trace, electrotonic potentials. In $B$ the current is twice that in $A$. Period of stimulation indicated by thickening of traces due to stimulus artifacts. $[K]_o = 10$ mM.

### *Changes in potassium concentration*

Too little, unfortunately, is known of the ionic composition of the salivary gland cells and of the relative permeabilities of the resting cell membrane to allow a detailed investigation of the ionic basis of the conductance change. However, the amplitude of the secretory potential is sensitive to the external potassium concentration (House, 1973) and it therefore seemed

of interest to see if this also affected the 'transmitter equilibrium potential'. Fig. 6 shows that it did. In the experiment where $[K]_o$ was 10 mM shown in *A*, the response was inverted by hyperpolarization and the 'transmitter equilibrium potential' was estimated as 54 mV more negative than the resting level. When the experiment was repeated after the bathing solution had been replaced by one in which $[K]_o$ was reduced to 1 mM the resting potential became more negative by 2 mV, the response was no longer inverted at the hyperpolarized level (Fig. 6*B*), and the 'transmitter equilibrium potential' was calculated to be $-111$ mV with respect to the new resting level. Thus, the net change in 'transmitter equilibrium potential' was $-59$ mV. In another experiment the change in 'transmitter equilibrium potential' was $-56$ mV for a tenfold change in $[K]_o$. The excellent agreement between these values and the theoretical change ($-58$ mV) that would be expected if the secretory potential were due entirely to an increase in potassium conductance is no doubt coincidental, but it strongly suggests that such an increase makes an important contribution to the response at its peak.

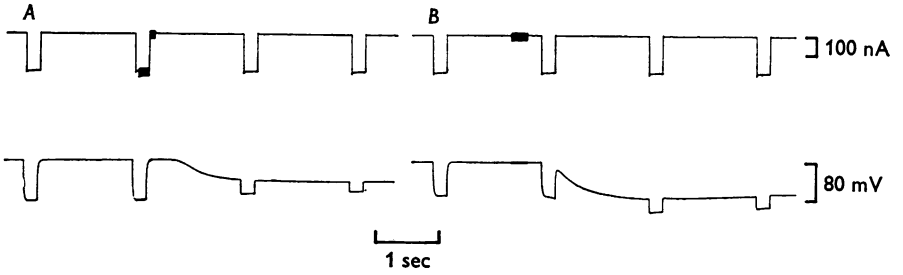


Fig. 6. Effect of potassium concentration. *A* and *B*: upper trace, current pulses; lower trace, electrotonic potentials. $[K]_o$ = 10 mM in *A* and 1 mM in *B*. Period of stimulation indicated by thickening of traces due to stimulus artifacts.

### *Comparison with dopamine response*

It has recently been suggested that the transmitter responsible for the secretory potential may be a catecholamine (Bland *et al.* 1973) possibly dopamine (House, Ginsborg & Silinsky, 1973). It was therefore of interest to compare the effect of dopamine with that of nerve stimulation. Fig. 7 shows the result of an experiment on two different acini in the same preparation, bathed in 10 mM-K. In *A*, the resting potential was $-22$mV and the 'transmitter equilibrium potential' was 39 mV more negative, i.e. $-61$ mV. In *B*, the resting potential was $-38$ mV and the 'dopamine equilibrium potential' was 27 mV more negative, i.e. $-65$ mV. In three other cells of the same preparation bathed in 5 mM-K the estimated equi-

librium potentials for the dopamine response were $-82$, $-91$ and $-94$ mV. The change in the equilibrium potential for a twofold change in $[K]_o$ is rather larger than the theoretical change (17 mV).

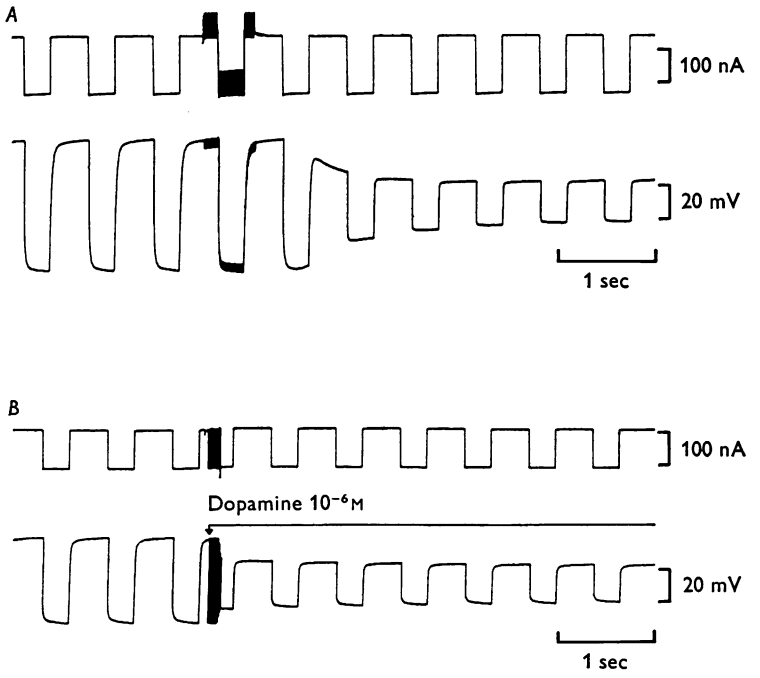


Fig. 7. Comparison of responses evoked by nerve stimulation and dopamine. *A*: upper trace, current pulses; lower trace, electrotonic potentials. Period of stimulation (50 stimuli at 100 Hz) indicated by thickening of traces due to stimulus artifacts. *B*: upper and lower traces as in *A*. Thickening of traces due to reduction of chart speed by a factor of 300.

## DISCUSSION

As stated in the introduction, the main object of the present experiments was to investigate the possibility of inverting the secretory potential, and the results clearly show that this can be done. Although for simplicity, the reversal potential has been referred to as the 'transmitter equilibrium potential' it is by no means certain that the two are identical. It will in fact be so only if the resistance between cells in the same acinus is small with respect to that between the interior and exterior. Until more is known about electrical coupling in the acinus, quantitative interpretations must be regarded cautiously. Thus although the possibility (House, 1973) that the main cause of the secretory hyperpolarization is an increase in potas-

sium permeability has been strengthened, the participation of other ionic changes has not been excluded. Indeed during the recovery to the resting potential, it seems that such changes must occur.

We would like to thank Mr Colin Warwick for preparing the illustrations. E. M. Silinsky would like to thank Professor E. W. Horton for his hospitality.

## REFERENCES

BERANEK, R., MARTIN, A. R. & WICKELGREN, W. O. (1970). Effects of iontophoretically applied drugs on spinal interneurones of the lamprey. *J. Physiol.* **207**, 653–665.

BLAND, K. P., HOUSE, C. R., GINSBORG, B. L. & LASZLO, I. (1973). Catecholamine transmitter for salivary secretion in the cockroach. *Nature, New Biol.* **244**, 26–27.

FRÖMTER, E. (1972). The route of passive ion movement through the epithelium of *Necturus* gallbladder. *J. Membrane Biol.* **8**, 259–301.

GAGE, P. W. & EISENBERG, R. S. (1969). Capacitance of the surface and transverse tubular membrane of frog sartorius muscle fibres. *J. gen. Physiol.* **53**, 265–278.

HOUSE, C. R. (1973). An electrophysiological study of neuroglandular transmission in the isolated salivary glands of the cockroach. *J. exp. Biol.* **58**, 29–43.

HOUSE, C. R., GINSBORG, B. L. & SILINSKY, E. M. (1973). Dopamine receptors in cockroach salivary gland cells. *Nature, New Biol.* **245**, 63.

IMAI, Y. (1965). Studies on the secretory mechanism of submaxillary gland of dog. *J. physiol. Soc. Japan* **27**, 304–312.

KATZ, B. (1966). *Nerve, Muscle and Synapse*, 1st edn, pp. 68–69. New York: McGraw-Hill Book Co.

LOEWENSTEIN, W. R. & KANNO, Y. (1964). Studies of an epithelial (gland) cell junction I. Modifications of surface membrane permeability. *J. cell Biol.* **22**, 565–586.

PETERSEN, O. H. (1970). The dependence of the transmembrane salivary secretory potential on the external potassium and sodium concentration. *J. Physiol.* **210**, 205–215.

THOMAS, R. C. (1972). Intra-cellular sodium activity and the sodium pump in snail neurones. *J. Physiol.* **220**, 55–71.

TRAUTWEIN, W. & DUDEL, J. (1958). Zum Mechanismus der Membranwirkung des Acetylcholin an der Hermuskelfasser. *Pflügers Arch. ges. Physiol.* **266**, 324–334.

WHITEHEAD, A. T. (1971). The innervation of the salivary gland in the American cockroach: light and electron microscopic observations. *J. Morph.* **135**, 483–505.

YOSHIMURA, H. & IMAI, Y. (1967). Studies on the secretory potential of acinal cells of dog's submaxillary gland and the ionic dependency of it. *Jap. J. Physiol.* **17**, 280–293.