J. exp. Biol. **118**, 427–431 (1985)
Printed in Great Britain © *The Company of Biologists Limited 1985*

SHORT COMMUNICATION

EXTRACELLULAR CHANGES IN ION CONCENTRATIONS INDUCED BY ACETYLCHOLINE IN THE SALIVARY GLANDS OF THE POND SNAIL *PLANORBIS CORNEUS*

BY ANDREW BARBER* AND GERRIT TEN BRUGGENCATE
Physiological Institute, University of Munich, Pettenkoferstrasse 12, 8000 Munich 2, West Germany

Accepted 20 March 1985

Salivary gland cells of the pond snail *Planorbis corneus* produce a biphasic depolarizing-hyperpolarizing electrical response following the application of acetylcholine (ACh). Ion-substitution experiments have indicated that the depolarizing phase of the ACh response is produced at least in part by an influx of Na^+, while the subsequent hyperpolarization results from the activity of an electrogenic Na^+/K^+ pump (Barber, 1985). It is not known, however, whether other species of ions also participate in this response, and the role of the extracellular medium as a source of the ions which compose the salivary secretion remains unclear. For these reasons the present study with ion-selective microelectrodes was undertaken in order to measure directly the shifts in extracellular ion concentrations which take place during stimulation with ACh.

Salivary glands were isolated from specimens of *Planorbis corneus* as previously described (Barber, 1985). Pairs of glands were transferred to a recording chamber (volume 0·2 ml) which was continuously perfused at a rate of 7·5 ml min^{-1} with snail saline at room temperature (20–28 °C). Normal physiological saline contained (in mmol l^{-1}) NaCl, 39; KCl, 1·3; $CaCl_2$, 4·5; $MgCl_2$, 1·5; $NaHCO_3$, 7·0; and was bubbled with a CO_2/O_2 gas mixture until it had a pH between 7·5 and 7·2. Ca^{2+}-free saline, made by substituting $MgCl_2$ for $CaCl_2$ and adding 1 mmol l^{-1} EGTA, was also used. AChBr (Sigma) and ouabain (Serva) were dissolved in these salines and perfused through the chamber for periods of 10 s and 2 min respectively.

Ion-selective microelectrodes were constructed as described by Grafe, Rimpel, Reddy & ten Bruggencate (1982). Briefly, micropipettes were pulled from theta capillaries and their tips were broken to a diameter of around 1–2 μm. The insides of the tips of the ion-selective barrels were then silanized with hexamethyl-

*Address for reprints: Abteilung Pharmakologie, E. Merck AG, Frankfurter Strasse 250, 6100 Darmstadt 1, West Germany.
Key words: Salivary glands, acetylcholine, ion-selective microelectrodes.

disilazane (Sigma) and filled with a small column of liquid ion exchanger. For K^+-, Na^+-, Cl^-- and Ca^{2+}-selective electrodes the ion exchangers used were valinomycin (Fluka), ETH 227 (Professor Simon, Zurich), IE-170 (W-P Instruments) and ETH 1001 (Professor Simon, Zurich) respectively. The shank and barrel of the ion-selective side of the electrode were then filled with 200 mmol l^{-1} KCl, NaCl, KCl or $CaCl_2$ for the K^+-, Na^+-, Cl^- and Ca^{2+}-selective electrodes respectively. Reference barrels were filled with 1 mol l^{-1} Mg-acetate in the case of the K^+-and Na^+-electrodes, 1 mol l^{-1} Mg-sulphate for Cl^--electrodes and 1 mol l^{-1} K-acetate for Ca^{2+}-electrodes.

K^+-electrodes were calibrated in solutions containing a constant background of 50 mmol l^{-1} NaCl and various concentrations of KCl and had a mean slope of 53 mV/decade K^+ change ($N=10$). Calibrating solutions for Na^+-electrodes consisted of 50 mmol l^{-1} KCl with different concentrations of NaCl; Na^+-electrodes had a slope of 53 mV/decade Na^+ change ($N=6$) and a Na:K selectivity ratio of 1:0·06. Cl^--electrodes were calibrated in KCl solutions (slope 52 mV/decade; $N=4$), while Ca^{2+}-buffered solutions, consitituted as described by Alvarez-Leefmans, Rink & Tsien (1981), were used to calibrate Ca^{2+}-electrodes (slope 28 mV/decade; $N=4$).

During experiments the tips of these electrodes were positioned between the folds of the gland so as not to be in contact with the gland surface. Intracellular recordings were made simultaneously from salivary cells in neighbouring acini with conventional microelectrodes. These electrodes were filled with 3 mol l^{-1} K-acetate and had resistances of from 10 to 50 MΩ. Attempts were made to record from the lumen of the gland by driving the ion-selective electrode through the cell layer, but these were without success. All ion movements reported here are expressed as concentration changes ±S.E. Conversions to ion activities were not made in the present study.

The depolarizing phase of the response to 10^{-4} mol l^{-1} ACh was associated with a rapid rise in the extracellular K^+ concentration ($[K^+]_o$), as shown in Fig. 1A. The magnitude of this increase varied with the position of the electrode, but averaged 0·6 ± 0·05 mmol l^{-1} ($N=47$). The ACh-evoked hyperpolarization, in contrast, was often accompanied by a small K^+ undershoot of the order of −0·1 ± 0·02 mmol l^{-1} ($N=24$). These changes in $[K^+]_o$ had a similar time course to the two phases of the membrane potential response to ACh.

The large increase in $[K^+]_o$ seen following the application of ACh would seem to indicate that ACh opens channels in the contraluminal membranes of the salivary cells which are permeable to K^+. It is also possible, however, that K^+ may leave salivary cells through voltage-dependent K^+-channels opened indirectly by the ACh-induced membrane depolarization, or through leakage pathways as a result of the increased driving force on K^+ during the depolarization.

The application of 10^{-4} mol l^{-1} ouabain produced a transient increase in $[K^+]_o$ (0·4 ± 0·15 mmol l^{-1}; $N=6$), after which $[K^+]_o$ returned slowly to its original level. The source of this K^+ remains to be determined, since K^+ could be released from, for example, presynaptic nerve terminals as well as gland cells. The most

noticeable effect of ouabain, however, was to produce an irreversible block of the hyperpolarizing phase of the ACh response, along with the abolition of the K^+ undershoot (Fig. 1A) in all six cases tested. This finding demonstrates that an electrogenic Na^+/K^+ pump is responsible for both the hyperpolarization and the re-uptake of K^+ from the extracellular space. Ouabain usually had little effect at first on either the ACh-induced depolarization or the concomitant increase in $[K^+]_o$, though both became gradually smaller with the repeated application of ACh. This is presumably a result of the reduced transmembranal K^+ gradient which should develop as K^+ leaves the salivary cells without being replaced.

The depolarizing action of 10^{-4} mol l^{-1} ACh coincided with a reduction in the extracellular Na^+ concentration ($[Na^+]_o$) by $-0{\cdot}6 \pm 0{\cdot}04$ mmol l^{-1} ($N=12$; Fig. 1B), probably because ACh opens Na^+ channels in the contraluminal membrane of gland cells and so leads to an influx of Na^+. An overshoot of $[Na^+]_o$ by around 0·2 mmol l^{-1} occurred in parallel with the hyperpolarizing phase of the ACh response in two of these cases. The application of 10^{-4} mol l^{-1} ouabain did not produce any detectable change in $[Na^+]_o$, but ouabain did abolish the ACh-induced overshoot (Fig. 1B). This indicates that the Na^+ overshoot is a consequence of the electrogenic extrusion of Na^+ from the salivary cells. ACh had no apparent influence on the extracellular concentrations of either Cl^- ($N=10$) or Ca^{2+} ($N=15$). Changes in extracellular Cl^- and Ca^{2+} as low as 0·5 mmol l^{-1} and 0·1 mmol l^{-1}, respectively, would have been detected in these experiments.

The biphasic membrane potential response of *Planorbis* salivary gland cells to ACh (Barber, 1985) is qualitatively similar to that described for cells in the mouse parotid and rat submandibular glands by Roberts & Petersen (1978). Furthermore, the ACh-evoked changes in $[K^+]_o$ and $[Na^+]_o$ in *Planorbis* closely resemble those measured by flame photometry in the extracellular fluid perfusing the cat submandibular gland following ACh stimulation (Petersen, 1970; Poulsen, 1974; Laugesen, Nielsen & Poulsen, 1976). These authors concluded that ACh increases the membrane permeability to both K^+ and Na^+, and so elicits a passive efflux of K^+ and an influx of Na^+. The subsequent re-uptake of K^+ and extrusion of Na^+ were both inhibited by ouabain (Petersen, 1970; Poulsen, 1974), as in *Planorbis*. Although it is not yet clear what type of channels permeable to K^+ are opened by ACh in the *Planorbis* gland, it seems possible that a similar mechanism operates for the ACh response in mammalian salivary glands and those of *Planorbis*.

While it remains to be demonstrated that ACh elicits secretion from the salivary glands of *Planorbis*, other electrically excitable gland cells, such as cultured primary anterior pituitary (Vale *et al.* 1978) and chromaffin (Suchard, Lattanzio, Rubin & Pressman, 1982) cells, do secrete when depolarized by, for example, K^+. An analysis of the salivary secretion has yet to be performed, but the present experiments indicate that Na^+ from the extracellular fluid should be available to be incorporated into saliva. It is relevant to note here that the production of primary secretory fluid in mammalian salivary glands also involves the transcellular movement of Na^+ (Young, 1979).

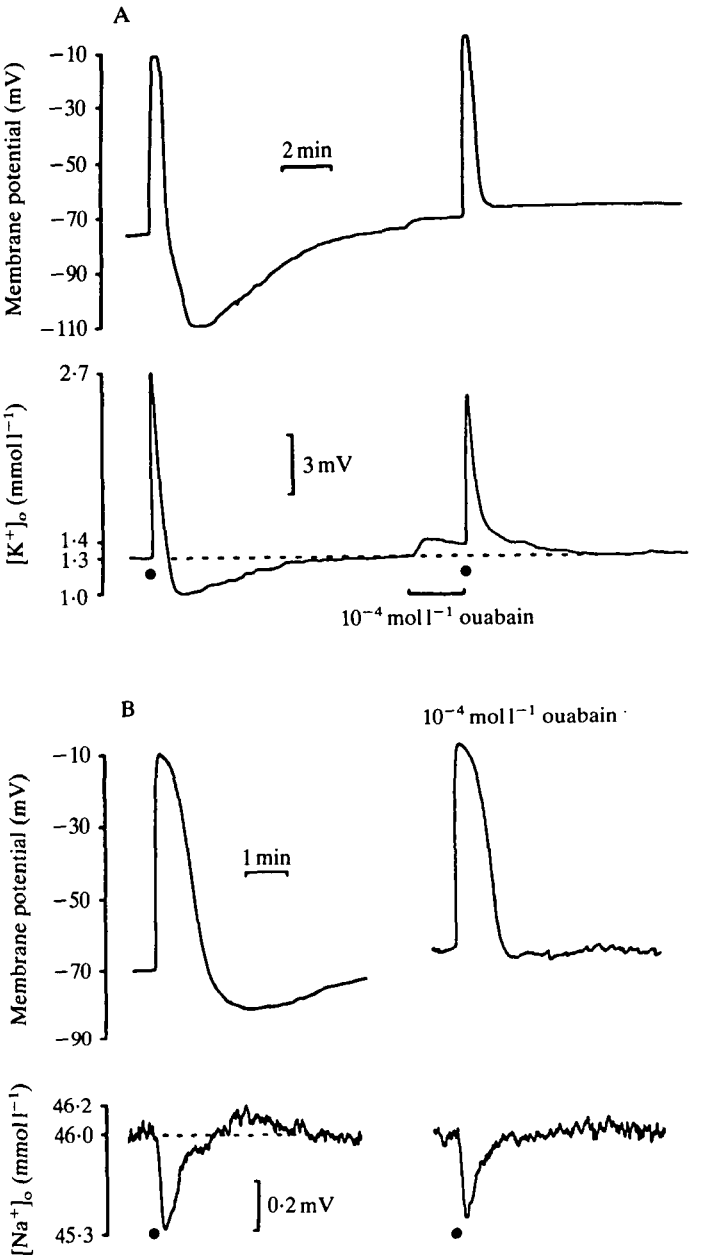

Fig. 1. ACh-induced changes in extracellular ion concentrations. In these figures the lower recordings were made with ion-selective electrodes positioned extracellularly, while the upper traces are intracellular recordings from salivary cells in neighbouring acini. The bath-application of 10^{-4} mol l^{-1} ACh for 10 s (marked by dots) elicits a biphasic change in (A) $[K^+]_o$ and (B) $[Na^+]_o$. Ouabain irreversibly blocks the ACh-evoked (A) $[K^+]_o$ undershoot and (B) the $[Na^+]_o$ overshoot. These experiments were performed in Ca^{2+}-free saline.

The absence of any detectable ACh-induced change in extracellular Ca^{2+} is of interest because an increase in the intracellular Ca^{2+} concentration is generally held to be a prerequisite for secretion (Case, 1984). The fact that no change in extracellular Ca^{2+} concentration was detected in the *Planorbis* salivary gland preparation following depolarization may mean that in this system ACh causes the release of Ca^{2+} ions from intracellular stores. The influx of Na^+ during the ACh-induced depolarization could be important in this respect because it has been proposed in other systems that Na^+ may release Ca^{2+} from bound pools (Lowe, Richardson, Taylor & Donatsch, 1976; Atwood, Charlton & Thompson, 1983) or decrease the ability of intracellular Ca^{2+}-sequestering sites to bind Ca^{2+} (Suchard *et al.* 1982; Case, 1984).

Financial support for this work was provided by the University of Munich. We thank Dr N. T. Slater for his critical comments on an earlier draft of this manuscript, and Professor Simon (Zurich) for generously donating ion-exchangers.

REFERENCES

ALVAREZ-LEEFMANS, F. J., RINK, T. J. & TSIEN, R. Y. (1981). Free calcium ions in neurones of *Helix aspersa* measured with ion-selective micro-electrodes. *J. Physiol., Lond.* **315**, 531–548.

ATWOOD, H. L., CHARLTON, M. P. & THOMPSON, C. S. (1983). Neuromuscular transmission in crustaceans is enhanced by a sodium ionophore, monensin, and by prolonged stimulation. *J. Physiol., Lond.* **335**, 179–195.

BARBER, A. (1985). Actions of acetylcholine on the salivary gland cells of the pond snail *Planorbis corneus*. *Comp. Biochem. Physiol.* **80C**, 175–184.

CASE, R. M. (1984). The role of Ca^{2+} stores in secretion. A workshop led by R. M. Case. *Cell Calcium* **5**, 89–110.

GRAFE, P., RIMPEL. J., REDDY, M. M. & TEN BRUGGENCATE, G. (1982). Lithium distribution across the membrane of motoneurons in the isolated frog spinal cord. *Pflügers Arch. ges. Physiol.* **393**, 297–301.

LAUGESEN, L. P., NIELSEN, J. O. D. & POULSON, J. H. (1976). Partial dissociation between salivary secretion and active potassium transport in the perfused cat submandibular gland. *Pflügers Arch. ges. Physiol.* **364**, 167–173.

LOWE, D. A., RICHARDSON, B. P., TAYLOR, P. & DONATSCH, P. (1976). Increasing intracellular sodium triggers calcium release from bound pools. *Nature, Lond.* **270**, 337–338.

PETERSEN, O. H. (1970). Some factors influencing stimulus-induced release of potassium from the cat submandibular gland to fluid perfused through the gland. *J. Physiol., Lond.* **208**, 431–447.

POULSEN, J. H. (1974). Acetylcholine-induced transport of Na^+ and K^+ in the perfused cat submandibular gland. *Pflügers Arch. ges. Physiol.* **349**, 215–220.

ROBERTS, M. L. & PETERSEN, O. H. (1978). Membrane potential and resistance changes induced in salivary gland acinar cells by microionophoretic application of acetylcholine and adrenergic agonists. *J. Membrane Biol.* **39**, 297–312.

SUCHARD, S. J., LATTANZIO, F. A., RUBIN, R. W. & PRESSMAN, B. C. (1982). Stimulation of catecholamine secretion from cultured chromaffin cells by an iontophore-mediated rise in intracellular sodium. *J. cell Biol.* **94**, 531–539.

VALE, W., RIVIER, C., YANG, L., MINICK, S. & GUILLEMIN, R. (1978). Effects of purified hypothalamic corticotropin-releasing factor and other substances on the secretion of adreno corticotrophin and β-endorphin-like immunoreactivities in vitro. *Endocrinology* **103**, 1910–1915.

YOUNG, J. A. (1979). Salivary secretion of inorganic electrolytes. *Int. Rev. Physiol.* **19** (ed. R. K. Crane), pp. 1–58.