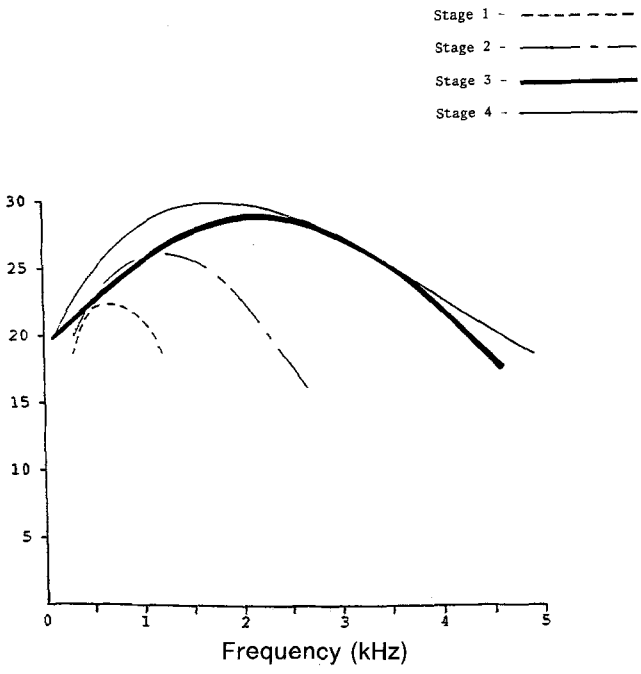

Figure 3. Frequency range patterns of associated stages (substrate-borne) of mating behaviour.

N. viridula that were electrophysiologically identified and classified by Cokl and Amon[8], in that the frequency bands represented in the figure are consistent with the frequencies detectable by the various interneurones.

We conclude that our results from an Australian population of *N. viridula* support an interpretation that substrate-borne rather than air-borne signals are the primary means of auditory sexual communication in this species.

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Amiloride impairs the cholinergic regulation of potassium permeability in the human sweat gland but not in the rat submandibular gland

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Abstract. Potassium permeability was monitored in human sweat glands and rat submandibular glands. Acetylcholine increased permeability in both tissues and the responses consisted of transient, calcium-independent and sustained, calcium-dependent components. Amiloride, a drug which inhibits $Na^+ - H^+$ countertransport, impaired the regulation of potassium permeability in sweat glands but not in the submandibular gland. It is suggested that the stimulus-permeability coupling process in the sweat gland may be sensitive to the lowering of internal pH.
Key words. Rat salivary gland; human sweat gland; amiloride; potassium permeability; $^{86}Rb^+$-efflux; stimulus-response coupling.

Acetylcholine increases intracellular free calcium ($[Ca^{2+}]_i$) in rodent salivary acini and primate sweat gland epithelia. This response, which is initiated by the release of calcium from cytoplasmic stores and is subsequently sustained by calcium influx, causes an elevation of cellular potassium permeability which is an important part of the secretory mechanism[1–5].

In the sweat gland the initiation of this permeability increase is acutely dependent upon external sodium ($[Na^+]_o$), suggesting that the receptor-regulated mobilisation of cytoplasmic calcium is $[Na^+]_o$-dependent[6]. In the submandibular gland, however, removal of sodium primarily inhibits the sustained component of the response indicating that, in this tissue, calcium influx may be sodium-dependent[7–9].

Removal of $[Na^+]_o$ thus exerts quite different effects upon the cholinergic regulation of potassium permeability in these two exocrine organs[6–9] and it has been sug-

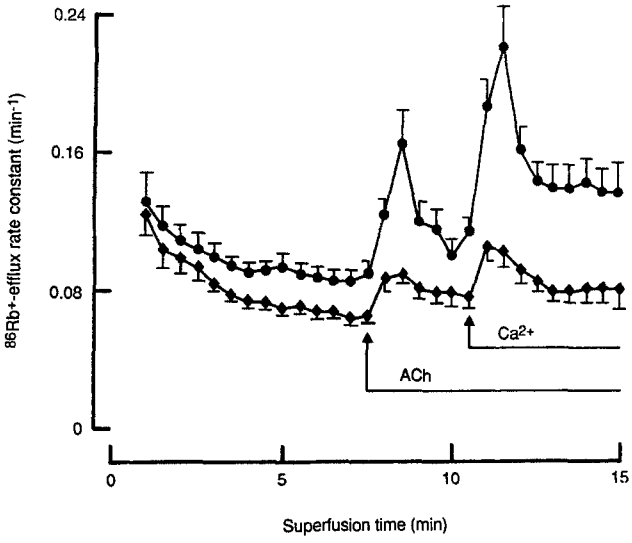
gested, for both tissues, that these effects of $[Na^+]_0$ removal may be secondary to the fall in internal pH (pH_i) which can develop if $[Na^+]_0$-dependent acid extrusion ($Na^+ - H^+$ exchange) is compromised[6-11]. If this is true then amiloride, a pharmacological inhibitor of $Na^+ - H^+$ exchange[12], should induce the same effects as removing $[Na^+]_0$ and so we have investigated the ability of this drug to impair the cholinergic regulation of potassium permeability in human sweat glands and rat submandibular glands.

Methods

Samples of human skin were obtained from the trunk region of patients undergoing general surgery; this procedure had the approval of the local Medical Ethics Committee and the informed consent of the patients. The specimens were placed in ice-cold saline (composition in mmol · l^{-1}: NaCl, 103; KCl, 4.7; $CaCl_2$, 2.56; $MgCl_2$, 1.13; $NaHCO_3$, 25; NaH_2PO_4, 1.15; D-glucose, 2.8; sodium fumarate, 2.7; sodium glutamate, 4.9; sodium pyruvate, 4.9) and then transported to the laboratory where 30–70 sweat glands were isolated[13] from each. Batches of 30–50 glands were then loaded with $^{86}Rb^+$ by superfusion for 1 h (2 ml · min^{-1}, 37 °C) with saline solution containing $^{86}RbCl$ (1–9 µmol · l^{-1}). Submandibular glands were obtained from freshly killed rats, placed in ice cold saline solution, chopped into pieces (< 0.5 mm) which were then incubated (30 min, 37 °C) in ^{86}Rb-containing saline (1–9 µmol · l^{-1}) and 30–50 mg of preloaded, glandular tissue was then transferred to a small flow cell.

The loaded tissues were then superfused (2 ml min^{-1}, 37 °C) with unlabelled saline solution. All tissues were initially washed for 2 min to remove external ^{86}Rb and sequential samples of chamber effluent were then collected at either 30-s (sweat glands) or 1-min (submandibular glands) intervals using a fraction collector. At the end of each experiment the $^{86}Rb^+$ content of each effluent sample and the residual $^{86}Rb^+$ content of the glandular tissue were determined by liquid scintillation counting. Rate constants for $^{86}Rb^+$-efflux were then calculated[1,3,9]. These methods have been described in detail elsewhere[1,3,9,13].

Tissues were initially superfused with a nominally calcium free solution to which acetylcholine (10^{-5} mol · l^{-1}) and calcium chloride (sweat glands, 2.56 mmol · l^{-1}, submandibular glands, 2 mmol · l^{-1}) were added sequentially. The effects of amiloride were studied by including this drug in the superfusing saline (1 mmol · l^{-1}) throughout the whole experiment. The unstimulated rate of $^{86}Rb^+$-efflux was calculated, for each experiment, as the mean efflux rate measured over the three samples collected immediately prior to addition of acetylcholine. Responses to acetylcholine were quantified by subtracting this basal efflux rate from that measured at the peak of the response. Responses to calcium were, similarly, quantified using the rate of efflux measured immediately prior to elevation of external calcium ($[Ca^{2+}]_0$). Data are presented as means ± standard errors, values of n refer to the number of subjects and the significance of any differences between means was determined using Student's unpaired t-test.



The regulation of potassium permeability in the human sweat gland. Rate constants for $^{86}Rb^+$-efflux have been plotted against superfusion time, data points are mean values and bars show the standard error of the mean. Glands were initially superfused with a nominally calcium free solution to which acetylcholine (10^{-5} mol · l^{-1}) and calcium chloride (2.56 mmol · l^{-1}) were added as indicated. Control data (•, n = 8) and data obtained in the presence of amiloride (♦, n = 7) are presented.

Comparison of the effects of amiloride upon the cholinergic regulation of potassium permeability in human sweat glands and in rat submandibular glands. Data are presented as means ± standard errors and values of n refer to the number of experiments. *, $p < 0.02$; **$p < 0.01$.

	Control	Amiloride
Human sweat gland (n)	8	7
Basal efflux rate constant (min^{-1})	0.088 ± 0.006	0.065 ± 0.004**
Ca^{2+}-independent response (Δmin^{-1})	0.077 ± 0.015	0.030 ± 0.004*
Ca^{2+}-dependent response (Δmin^{-1})	0.125 ± 0.023	0.039 ± 0.007**
Rat submandibular gland (n)	14	12
Basal efflux rate constant (min^{-1})	0.027 ± 0.002	0.025 ± 0.001
Ca^{2+}-independent response (Δmin^{-1})	0.025 ± 0.002	0.019 ± 0.003
Ca^{2+}-dependent response (Δmin^{-1})	0.026 ± 0.004	0.028 ± 0.003

Results

Sweat glands. Acetylcholine normally evoked an immediate increase in the rate of $^{86}Rb^+$-efflux during superfusion with the calcium free solution. This response was essentially transient but subsequent elevation of $[Ca^{2+}]_0$ evoked a further increase in efflux which was much more sustained (fig.). The normal response to acetylcholine thus consists of transient, $[Ca^{2+}]_0$-independent and sustained, $[Ca^{2+}]_0$-dependent phases.

Also presented in the figure are data obtained in the presence of amiloride. In the presence of this drug the basal rate of $^{86}Rb^+$-efflux was significantly slower than control and, although acetylcholine and calcium could still evoke increased $^{86}Rb^+$-efflux, the responses were significantly smaller than control (table).

Submandibular glands. Sequential addition of acetylcholine and calcium to the superfusing saline normally evoked transient and sustained increases in $^{86}Rb^+$-efflux (table) which were essentially identical to those presented previously[9]. In contrast to the sweat gland, however, the presence of amiloride did not significantly affect the unstimulated rate of $^{86}Rb^+$-efflux or either component of the response to acetylcholine (table).

Discussion

The rate of $^{86}Rb^+$-efflux from preloaded exocrine cells provides an indicator of potassium permeability only if the $^{86}Rb^+/K^+$ ratio is low[1,14]. We have previously shown that the experimental protocols used in the present study satisfy this condition[3,6,9]. Our control data thus confirm that acetylcholine increases potassium permeability in human sweat glands and in rat submandibular glands; in both tissues the responses normally consist of transient and sustained phases[3,9] that are attributable to the mobilisation of a limited cytoplasmic calcium pool and to receptor-regulated calcium influx respectively[1].

The present data show clearly that amiloride impairs the regulation of potassium permeability in the human sweat gland but that it does not do so in the rat submandibular gland. Amiloride can cause lowering of pH_i in exocrine acini[10,11] and this inhibits the large conductance, voltage- and $[Ca^{2+}]_i$-activated potassium channels found in cultured sweat gland epithelia[15]. This effect may explain why amiloride lowered potassium permeability in unstimulated sweat glands and reduced the response to acetylcholine. Similar channels are, however, present in submandibular acini[16] and so it is not immediately apparent why amiloride does not affect potassium permeability in this tissue. The potassium channels in submandibular acini[16] appear to be more sensitive to $[Ca^{2+}]_i$ than do those in sweat gland epithelia[15]. Furthermore, the potassium channels in *Necturus* gall bladder epithelia, which have a very similar $[Ca^{2+}]_i$-sensitivity to those in submandibular acini, appear to be much less sensitive to fall in pH_i than those in sweat gland cells[15-17]. The possibility that the potassium channels in sweat gland epithelia and submandibular acini may be differentially sensitive to pH_i thus cannot be excluded.

Calcium mobilising agonists, such as acetylcholine, can also evoke increased Na^+-H^+ exchange and so contribute to the control of pH_i in stimulated cells[18-20]. It has been reported, for human platelets, that the thrombin-evoked mobilisation of cytoplasmic calcium is acutely inhibited if this stimulation of acid extrusion is prevented, either by removal of $[Na^+]_0$ or by pharmacological blockade. It has therefore been proposed that this stimulation of Na^+-H^+ exchange is centrally important to the stimulus-response coupling[18]. This hypothesis could account for the $[Na^+]_0$-dependence seen in the human sweat gland as the responses of this tissue were essentially normal when $[Na^+]_0$ was replaced by lithium[6], a cation which can support Na^+-H^+ exchange[11]. It is possible that the sensitivity to amiloride which we now report may also, at least in part, be a due to such an action.

There is, however, strong evidence against such a central role for Na^+-H^+ exchange in stimulus-response coupling[20] and there is no evidence of such an effect in the submandibular gland. In many cell types pH_i and $[Ca^{2+}]_i$ are inversely related but there are also much less clearly understood interrelationships between these parameters and the internal sodium concentration ($[Na^+]_i$)[21,22]. The effects of simultaneous changes in $[Na^+]_i$ and pH_i upon $[Ca^{2+}]_i$ are therefore very hard to predict. Furthermore such manoeuvres could have different effects in different cell types if there were slight differences in $[Ca^{2+}]_i$ or pH_i buffering[21,22]. In this context it is interesting that the cholinergic regulation of potassium permeability in the sweat gland is impaired by removal of bicarbonate[6] whereas the responses of the submandibular gland are essentially normal under such conditions[9]. Removal of bicarbonate, in common with exposure to amiloride, can impair the regulation of pH_i[10,11] and so the present data, together with our earlier findings[6,9], may indicate that stimulus-response coupling in the sweat gland is strictly dependent upon the regulation of optimal pH_i.

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