the mechanisms of these changes.

Aldosterone was injected into the lymph sacs of frogs (Rana temporaria, 70 μg per frog) which were then placed in individual tanks containing running deionized water, overnight. Afterwards the ventral skin was dissected free and the stratum corneum peeled off. Animals which had already shed the stratum corneum were rejected. The skins were mounted horizontally in perspex cells for voltage clamping at zero potential and for labelling experiments. [^{14}C]-amiloride was used to label sodium channels in the mucosal surface of the skins, as described previously (Cuthbert, 1973).

It was confirmed that newly moulted skins were less sensitive to the blocking actions amiloride, although the sensitivity increased to normal ($Km = 2 \times 10^{-7}$ M, for skins bathed in solutions containing 111 mEq/l Na^+) within 4 hours. Omission of calcium from the mucosal bathing solution increased the insensitivity to amiloride in newly moulted skins, and delayed the recovery of sensitivity. Since treatment of normal skins with EGTA-Ringer reduces or abolishes the effect of amiloride (Cuthbert & Wong, 1972) it is considered that interaction of calcium with newly exposed (or induced) sodium channels is, at least, part of the mechanism involved before the blocking action of amiloride can be fully developed.

The number of sodium channels, measured in the presence of 1.1 mEq/l Na^+, in newly moulted skins was $712 \pm 59/\mu m^2$ (four determinations), a significant increase ($P < 0.05$) on the number for normal skins ($201 \pm 14/\mu m^2$, 15 determinations). Treatment of normal skins with EGTA in the absence of calcium and in the presence of 1.1 mEq/l Na^+ did not reduce the amount of amiloride bound. For example, in one group of normal skins exposed to ^{14}C-amiloride (10^{-8} M) the amount bound was 0.1 ± 0.01 pmole/9.6 cm^2 (four determinations) and the mean inhibition of transport was $22.9 \pm 1.4\%$. In the absence of calcium the amount bound was 0.15 ± 0.01 pmole/9.6 cm^2 (seven determinations) while transport was inhibited by only 4%. In four skins which moulted spontaneously the amount of amiloride bound was 0.44 ± 0.03 pmole/9.6 cm^2, in the absence of calcium.

The observations suggest that the increase in sodium transport following moulting is due to an increase in the density of sodium channels in the mucosal surface. Whether this results from uncovering occluded channels, or by induction of new channels by aldosterone is not yet clear.

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Voltage-clamp experiments on the potential-dependent behaviour of membrane ion channels operated by the muscarinic receptor of smooth muscle

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Stimulation of the muscarinic receptor increases the conductance of the cell membrane of smooth muscle (Bolton, 1972). The present experiments were done to discover if the additional conductance which appears when the muscarinic receptor is activated, varies as the membrane potential is charged.

Experiments were done on strips of smooth muscle either from guinea-pig uterus (after previous treatment with 200 μg oestradiol benzoate) or from guinea-pig ileum. The strips were 5 mm x 50-100 μm x 100-300 μm in size and were introduced into a double sucrose-gap apparatus (Rougier, Vassort & Stämpfli, 1968). In this, potential is recorded across one sucrose-gap (width 0.6-0.8 mm) while current can be passed across the other. The portion of active tissue ('node') between the sucrose gaps was 100-200 μm wide. Nodal potential was clamped using a conventional voltage-clamp circuit.

After clamping at the resting membrane potential, rectangular potentials of different sizes were imposed either in a hyperpolarizing or in a depolarizing direction. The currents required to clamp the potential at values positive to the resting membrane potential were changing only slowly

after 200 ms (ileum) or 2 s (uterus). These values of the current were used to plot the current-voltage relationship. Currents required to hyperpolarize the membrane were constant after the capacitive transient.

The current-voltage characteristics of the additional conductance appearing in the presence of carbachol were derived from the current-voltage plots obtained in the absence and in the presence of carbachol (10^{-7} to 10^{-5} g/ml), (Ginsborg, 1967). This conductance was constant for small deviations from the resting membrane potential but with large deviations it apparently increased. The current through this conductance reversed in direction at a point somewhat negative to the peak of the evoked spike. The position of this point, the equilibrium potential, was in good agreement with previous predictions (Bolton. 1972).

A further series of experiments were done using ramp commands (Fishman, 1970), since large rectangular command pulses may produce appreciable shifts in ion concentrations. Ramps have the additional advantage that if a suitable rate of rise is chosen, one or two applications of the ramp can yield the whole current-voltage curve. Using such ramps, the carbachol dependent conductance was found to change much less with large perturbations from the resting membrane potential.

Experiments have also been done on a short 'closed cable' preparation of smooth muscle using intracellular recording of membrane potential with microelectrodes. This 'closed cable' preparation was polarized uniformly by current passed into it from an external electrode. The preparation thus avoids the difficulties encountered in measuring the current-voltage characteristics of the usual 'infinite cable' preparation (Tomita, 1966) which arise due to the spatial decrement of polarization with distance from the current passing electrode.

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The effect of the dissociative anaesthetic, ketamine, on transmembrane potentials of Purkinjé fibres of the pig heart

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Several investigators have reported effects on the heart of the dissociative anaesthetic ketamine including depression (Goldberg, Keane & Phear, 1970), stimulation (Traber, Wilson & Priano, 1968) and antiarrhythmic activity (Goldberg *et al.*, 1970; Dowdy & Kaya, 1968). In this laboratory we have demonstrated that ketamine potentiates and then depresses the isolated rat diaphragm preparation (Hamilton, Jones, Kiraly & Parker, 1972) and that this was a direct effect upon the muscle, as both potentiation and blockade of the directly stimulated muscle were produced in the presence of tubocurarine. It was concluded that the initial stimulant actions were possibly veratrine-like in nature.

In the present investigation the actions of ketamine have been further studied on the Purkinjé conduction system of the mammalian heart. Intracellular recordings have been made from isolated spontaneously active and electrically-driven cells of the septomarginal trabecula (moderator band) of the pig.

Domestic pigs weighing between 4.5 and 9.0 kg were stunned with a captive bolt pistol, exsanguinated and the heart was rapidly removed. The moderator band was dissected out and placed in cold, oxygenated Krebs-Henseleit solution, and then transferred to an organ bath, held firmly by two threads placed over the pieces of myocardium at the ends of the band, and totally immersed in a continuous flow of pre-warmed ($37^{\circ} \pm 1^{\circ}$C) Krebs-Henseleit solution gassed with 95% oxygen, 5% carbon dioxide.

The preparation was then rested for a period of