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ACTION OF TANNIC ACID ON FROG MUSCLE

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Abstract—Tannic acid, when applied to frog sartorius muscles (10^{-8}–10^{-4} M) caused a depolarization. High concentrations (10^{-6}–10^{-4} M) produced a more rapid and greater fall in membrane potential. Membrane resistance, measured concurrently, showed an initial rise (at 10^{-7} and 10^{-6} M) and subsequently fell. It is suggested that these effects are the result of the action of tannic acid on the chloride permeability of the muscle membrane, and comparisons are made with the effect of this agent on red cells and on crayfish giant axon.

TANNIC acid has a number of effects on the surface properties of erythrocytes, but perhaps its major interest lies in the rapid reduction in anion permeability which it causes at low concentration.[1–3] Like many agents that affect the anion permeability of red cells,[4] it appears to have only a limited effect on the entry of cations, whilst the active efflux of Na^+ is unaffected (Radcliffe, Duncan and Bowler, unpublished). Membrane enzymes of red cells that are affected by tannic acid, and might therefore be implicated in the control of anion permeability, are the acetylcholinesterase,[5,6] and the Mg^{2+}- and Na^+-K^+-Mg^{2+}-ATPases (Radcliffe, Duncan and Bowler, unpublished).

Tannic acid is able to cross-link and "solidify" protein monolayers,[7] and if, as has been suggested, a conformational change in a membrane protein is involved in excitation,[8–11] then tannic acid might be expected to produce radical alterations in the electrical responses of nerves.[7,12] However, application of tannic acid (2×10^{-6}–10^{-4} M) to the outer surface of crayfish axons for 40 min was without effect in altering their electrical properties. When tannic acid was perfused internally (10^{-5} M), spontaneous activity was initiated, with a progressive increase in spike width. The action potentials were up to several minutes in duration after perfusion for 30 min and it has been suggested that these changes are caused by a maintained increase in sodium conductance.[11]

There is a relatively low passive Cl^- permeability in crayfish giant axon, the ratio P_{Cl}/P_K being only 0·13 (ref. 13), and it is probable that this ion, as in squid nerve, is relatively unimportant in determining the resting potential of the crayfish axon. In frog muscle, however, although the resting potential is again dependent on K^+ and Na^+ (ref. 14), Cl^- permeability is also high. Cl^- conductance is about twice the K^+ conductance in resting muscle, the chloride ions being passively distributed. Since the action of tannic acid on the red cell membrane is predominantly a reduction of anion permeability, the action of this agent on the electrical properties of frog muscle has been tested.

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METHODS

The frog saline used contained: NaCl 111·0 mM; KCl 1·88 mM; $CaCl_2$ 1·1 mM; $NaHCO_3$ 1·2 mM; glucose 11·0 mM; pH 7·3 at 20°. Tannic acid was obtained from BDH, Poole, England; all inorganic salts were AnalaR grade. Since tannic acid has a high affinity for clean glass,[2] all glassware was siliconed and equilibrated with tannic acid solutions of appropriate concentration. All solutions of tannic acid were prepared immediately before use.

Microelectrodes were filled with 3 M KCl by the method of Tasaki *et al.*[15] from Jencons H15/10 microelectrode capillary tubing and were examined for tip potentials before use by the method of Adrian.[16] Any with tip potentials greater than 10 mV were discarded. Microelectrode resistance was measured by the method of Frank and Fuortes.[17] Membrane resistance was measured by the method of Schwanne, Kawata, Schäfer and Lavallée,[18] using a Tektronix 502A oscilloscope and an Advance A.F. Signal generator (type H1). Sartorius muscles from frog (*Rana temporaria*) were used, mounted in a chamber of 12·5 ml capacity.

RESULTS

In all experiments, surface fibres were successively impaled and the membrane resistance and potential determined, enabling readings to be made at intervals of about 1–2/min. This method of making multiple readings was chosen to eliminate errors due to DC drift. The results are shown in Figs. 1–3.

Previous experiments have shown that the effect of tannic acid is related both to the concentration of the solution and to the number of μmoles tannic acid per unit weight of protein exposed. A standard volume of 12·5 ml tannic acid solution was used in these experiments, but small variations in the effect of very weak tannic acid solutions

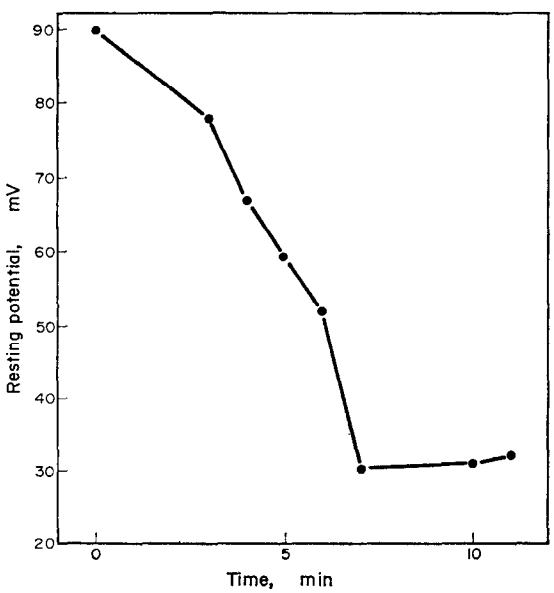

FIG. 1. The action of 10^{-4} M tannic acid on the membrane potential of frog sartorius muscle. Ordinate: resting potential in mV. Abscissa: time in minutes. No resting potential was recorded after 15 min.

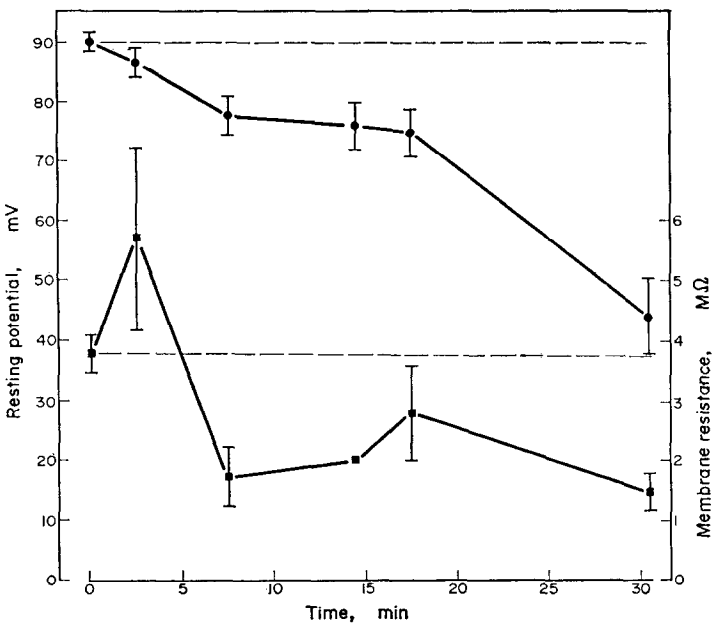

FIG. 2. The action of 10^{-6} M tannic acid on frog sartorius muscle. Upper line (——— ● ———): resting potential in mV. Lower line (——— ■ ———): membrane resistance in MΩ, method of measurement as ref. 18. Vertical bars represent the S.E. of the mean, $n = 10$ for each observation. The dashed lines represent mean values of potential and resistance before application of tannic acid ($n = 30$).

were noted. Nevertheless, a very consistent pattern emerged. At 10^{-4} M tannic acid (Fig. 1), the muscle depolarized rapidly, the resting potential fell to −27 mV in 10 min and the muscle was then inexcitable. The resting potential falls more slowly at 10^{-6} M, reaching about −40 mV after 30 min and the surface fibres were inexcitable after 35 min. Deep fibres in the muscle, however, were still excitable at this time. Membrane resistance, however, rose during the first 5 min after exposure, but thereafter fell steadily, becoming immeasurably low after 35 min (Fig. 2).

At 10^{-7} M tannic acid the resting potential fell from −90 mV to about −70 mV over 20 min and thereafter showed a gradual and partial recovery. When the tannic acid solution was renewed after this time, a second fall in resting potential was seen, followed by a second partial recovery (Fig. 3). Associated with these changes in resting potential, the membrane resistance again showed an initial rise of 12–15 min duration, after which it returned and remained at a normal level. Renewing the tannic acid solution after 30 min at 10^{-7} M again produced an initial rise in membrane resistance which returned to normal after about 10 min. These initial rises in membrane resistance were consistently obtained at 10^{-7} and 10^{-6} M tannic acid (Figs. 2 and 3). Only a small fall in resting potential was seen with 10^{-8} M tannic acid, and no significant changes in membrane resistance occurred. Excitability was apparently unimpaired after 90 min at 10^{-8} M.

DISCUSSION

The effect of tannic acid on frog muscle is clearly different from its action on the crayfish giant axon. The latter preparation was insensitive to external application, but

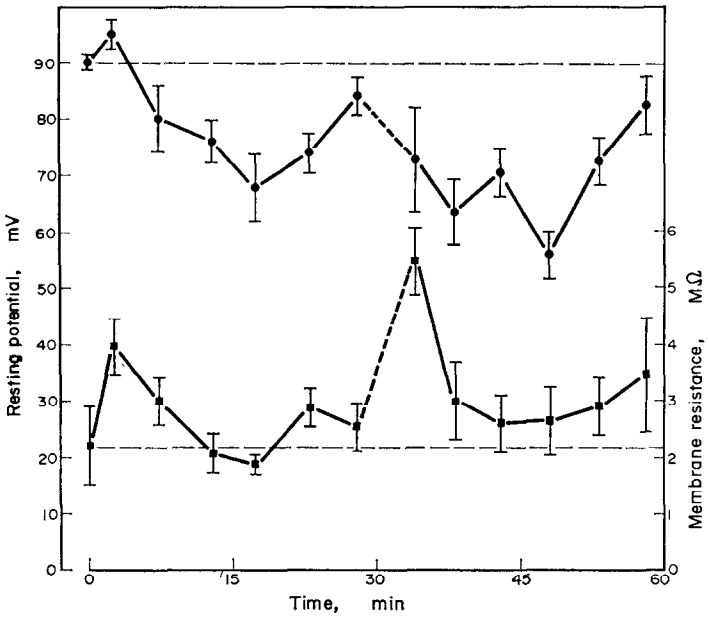

FIG. 3. The action of 10^{-7} M tannic acid on frog sartorius muscle. Upper line (——— ● ———): resting potential in mV. Lower line (——— ■ ———): membrane resistance in MΩ, method of measurement as ref. 18. Vertical bars represent the S.E. of the mean, $n = 20$ for each observation. Horizontal dashed lines represent mean values of potential and resistance before application of tannic acid ($n = 30$). Broken line shows point of renewal of tannic acid solution.

continuous perfusion of the axons with solutions of 3–16 $\times$ 10^{-6} M produced a fall in the resting potential of 10–30 mV after 10 min. During this depolarization, spontaneous activity often appeared and the spike width progressively increased.[7,12] Frog muscle, on the other hand, has been shown to be sensitive to the single, external application of much more dilute solutions of tannic acid; high concentrations of this agent produced a maintained depolarization, but at 10^{-7} M the resting potential recovered after an initial fall. It is difficult to account for these differences between the results obtained with crayfish axon and with frog muscle, although the rapid depolarization observed in these experiments at 10^{-4} M may well be the result (as in crayfish axon) of an increase in Na^+ permeability.

Frog muscle is permeable to Cl^- as well as to K^+, the chloride conductance being about twice that of potassium in the resting muscle,[14] and the resting potential can be specified:

$$V = \frac{RT}{F} \ln \frac{P_K [K]_o + P_{Na} [Na]_o + P_{Cl} [Cl]_i}{P_K [K]_i + P_{Na} [Na]_i + P_{Cl} [Cl]_o}.$$

However, Cl^- is not actively transported in frog muscle and becomes distributed in accord with the Donnan equilibrium; P_{Cl} is independent of both the Cl^- concentration and the membrane potential.[19] The membrane potential is normally determined by the Na^+ and K^+ concentration gradients and permeabilities. Changing $[Cl]_o$, whilst keeping $[K]_o$ constant, therefore, produces only a temporary alteration of membrane

potential, while the new equilibrium is being established. When $[Cl]_0$ for single frog muscle fibres was suddenly reduced from 120 to 30 mM, the membrane depolarized rapidly by some 20 mV and recovered slowly over the following 10 min.[14] Such a depolarization and recovery superficially resembles the effects on the resting potential which are observed with 10^{-7} M tannic acid (Fig. 3). Furthermore, the action of tannic acid on red cells is primarily to cause a marked reduction in anion permeability[2,3] and all the available evidence suggests that tannic acid acts on the external surface when applied to cells and does not cross the plasma membrane.[2,4–6,20] Thus, it is possible that tannic acid specifically affects the permeability of the frog muscle membrane for the inward movement of Cl^-, thereby altering the $(P_{Cl} \times [Cl]_0)$ term in the equation above, with a consequent depolarization of the membrane which is maintained until the new equilibrium is established. Such an explanation would be in accord with the observed initial increase in membrane resistance.

At 10^{-6} M tannic acid the membrane depolarized by about 15 mV but failed to recover and after 20 min the resting potential fell progressively. The initial rise in membrane resistance was followed by an increase in conductance. At these higher concentrations of tannic acid, therefore, there may be a progressive, direct increase in Na^+ conductance or, alternatively, the initial depolarization may cause an associated increase in Na^+ permeability. Frog sartorius muscle fibres, for example, become spontaneously active when depolarized by immersion in Cl^- free saline.

Intracellular Cl^- concentrations in crayfish axons are higher than would be expected from equilibrium considerations[21] and may be associated with an inwardly directed, active transport of Cl^- (ref. 22). However, there is a relatively low passive Cl^- permeability (the ratio P_{Cl}/P_K is only 0·13, ref. 13); so that variations in $[Cl]_0$, or a reduction in P_{Cl} will not have a significant effect on the resting potential. Thus, if the major action of tannic acid at low concentration is to affect Cl^- permeability it is probable that it will not markedly modify the electrical properties of crayfish axons when externally applied. It seems probable that cells that are particularly sensitive to tannic acid are those in which Cl^- permeability is high.

Frog muscle may, therefore, prove to be a useful preparation for the study of the action of tannic acid on anion permeability. Its advantage over mammalian red cells is that it permits the continuous monitoring of changes in membrane conductance and potential.

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