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The antagonism between salicylate-induced and pH-induced changes in the membrane conductance of molluscan neurons

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SUMMARY

The membrane potential and conductance of identified invertebrate neurons decreased with decreasing pH (9.3–4.8), due primarily to a decrease in the K^+ conductance of the membrane. Salicylate antagonized these effects by increasing K^+ conductance and decreasing Cl^- conductance. Since a local decrease in pH can evoke a painful response in animals and man, it is possible that salicylate's analgesic ability derives from its ability to antagonize the changes in membrane conductance produced by pH changes.

A decrease in external pH decreases K^+ permeability and increases Cl^- permeability in a variety of nervous tissues[1–3]. We have recently reported that the non-narcotic analgesic salicylate increases K^+ permeability and decreases Cl^- permeability of invertebrate neurons[4]. We now report the effects of changing external pH on the electrical properties of identified molluscan neurons and the antagonism between salicylate and pH, in influencing K^+ and Cl^- permeability.

The buccal ganglion from the marine mollusc *Navanax inermis* was isolated, mounted in a 5-ml lucite chamber and perfused with salt solution at room temperature (22–25°C). The salt solution contained 492 mM NaCl, 10 mM KCl, 11 mM $CaCl_2$, 50 mM $MgCl_2$, and 10 mM Tris–maleate–NaOH at pH 7.8 used as a buffer. An additional 100 mM $MgCl_2$ was added to the bathing solutions to reduce synaptic activity and thereby allow more accurate determination of the membrane potential and resistance. The

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osmolarity of this solution was 1300 mosM. The capsule enveloping the ganglion was opened and identified neurons impaled with double-barrel microelectrodes filled with 3 M KCl (2–10 MΩ resistance). This report is based on observations from 30 experiments on the cells previously identified[5]. Electrical potentials were amplified and displayed using standard techniques.

The pH of the salt solution was varied over a range of 4.8 to 9.3 using a Tris–maleate–NaOH buffer system. A decrease in pH from 7.8, the normal value, to 6.8 caused a reversible decrease in the membrane potential of all cells studied, if the exposure was less than 5 min (Fig. 1A, top trace). More prolonged exposure caused a progressive deterioration of the cell through an irreversible decrease in membrane potential. The effects of decreases in pH down to 4.8 were all reversible if the exposure time was less than 5 min. A fall in pH to 6.8 produced either a slight increase in input resistance or no detectable change (Fig. 1A, top trace). The time course of the potential change in response to a decrease in pH usually required 4–5 min to develop completely. All the data to be described was obtained within 5 min of exposure to a change in the pH of the bathing solution. A rise in the pH of the saline increased membrane potential and effective conductance (Fig. 1A, bottom trace). Over the range of pH studied, the membrane potential and conductance decreased with decreasing pH of the medium (Fig. 1B).

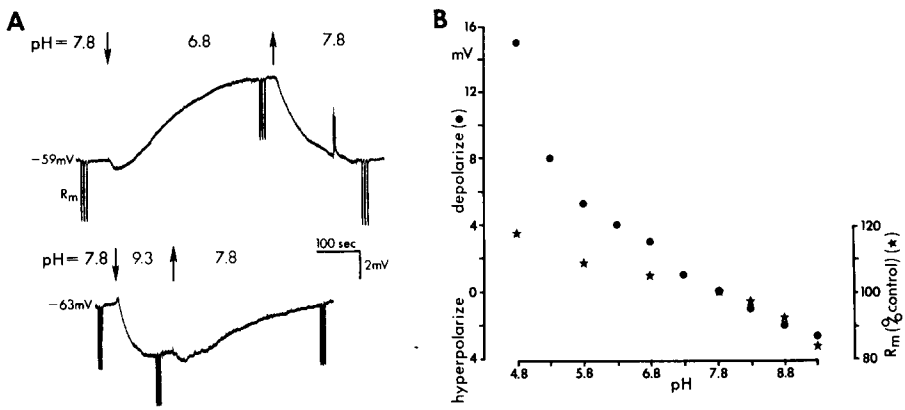

Fig. 1. The effect of pH on the membrane potential and input resistance. A. Top trace, the input resistance of the cell G-R was obtained at a resting potential of −59 mV by passing 4-nA pulses, 800 ms long (R_m) through a second intracellular electrode. Exposure to medium of pH 6.8 (downward arrow) caused a 5 mV depolarization with little change in input resistance. (The initial small hyperpolarization is due to a junctional potential.) Washing with medium at pH 7.8 (upward arrow) resulted in a return to control values. Bottom trace, the input resistance of the cell G-L was obtained at a resting potential of −63 mV by passing 5-nA pulses, 800 ms long through the second intracellular electrode. Upon exposure to medium of pH 9.3 (downward arrow) the cell hyperpolarized 3 mV, coincident with a decrease in input resistance. The membrane potential and resistance returned to control values upon washing with medium of pH 7.8 (upward arrow). B. The membrane potential and resistance are plotted as a function of external pH. These points are averages from the results of all cells studied. The ordinate to the left indicates the change in membrane potential from that at pH 7.8 (•), while the ordinate to the right shows the input resistance as a percentage of that at pH 7.8 (stars). Both the membrane potential and conductance decrease as the external pH decreases from 9.3 to 4.8.

In order to elucidate the ionic mechanisms involved in the response to variations in pH, we changed the external concentration of either K^+, Na^+, or Cl^- ($[K^+]_o$, $[Na^+]_o$, or $[Cl^-]_o$) at pH 7.8 and at lower pH values. External K^+ concentration was changed isosmotically by substituting NaCl for KCl. In salt solution with a low Na^+ concentration, 482 mM NaCl was replaced by 241 mM $MgCl_2$ and 234 mM mannitol, giving a solution containing 10 mM Na^+, with a constant concentration of Cl^-. The osmolarity of this solution was 1150 mosM. In salt solution with a low concentration of Cl^-, NaCl was replaced by sodium isethionate and $MgCl_2$ by $MgSO_4$ so that the final concentration of Na^+ remained constant while $[Cl^-]_o$ decreased to 22 mM. The osmolarity of this solution was 1250 mosM.

A comparison of the variation in membrane potential with $[K^+]_o$ at pH 7.8 and 5.8 (Fig. 2) indicates that reducing the pH from 7.8 to 5.8 depolarized the membrane potential over the 1–20 mM range of $[K^+]_o$ and extended the range over which the sensitivity to changes in $[K^+]_o$ was low. At pH 7.8 the slope of the membrane potential as a function of $[K^+]_o$ began to increase at about 8–10 mM $[K^+]_o$, while at pH 5.8 this did not occur until 20–30 mM $[K^+]_o$. The effect of pH on the membrane potential was small above 30 mM $[K^+]_o$.

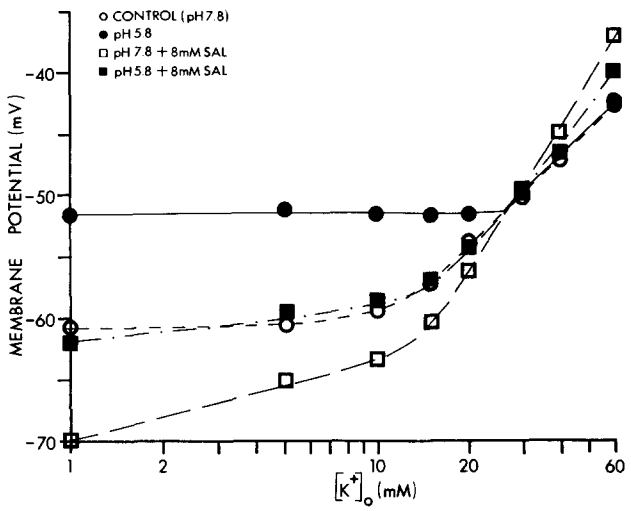

Fig. 2. The variation of membrane potential with $[K^+]_o$ is shown as a function of pH in the presence and absence of salicylate (SAL). The curve is shifted in a depolarizing direction over the 1–20 mM $[K^+]_o$ range when the pH of the saline is reduced from 7.8 (○) to 5.8 (●). The normal variation of membrane potential with $[K^+]_o$ over the 8–20 mM $[K^+]_o$ range is markedly reduced at pH 5.8. Addition of 8 mM salicylate to the medium of low pH results in a restoration of the curve to its normal configuration (■). The acidity of the medium has partially antagonized the effects of salicylate on K^+ permeability since the curve in salicylate at pH 5.8 lies between the control curve and the curve in salicylate at pH 7.8 (□).

The transient, 10–15 mV depolarizations following reductions in $[Cl^-]_o$ did not change in medium of pH 5.8. Our inability to detect an increase in Cl^- conductance might be explained by (1) an increased permeability of the Cl^- substitute (isethionate) under conditions of low pH and/or (2) the depolarized state of the membrane potential, which would reduce the apparent size of the transient. Since the change in input resistance of the membrane in response to a decrease in pH was quite small, there may have been an undetected increase in Cl^- conductance. The transient, 2 mV hyperpolarizations produced by decreasing $[Na^+]_o$ to 10 mM were unchanged by reducing the pH of the saline to 5.8. Therefore, the depolarization produced by bathing with a low pH medium was due primarily to a decrease in the K^+ conductance of the membrane.

Application of salicylate to pH-depolarized cells caused a rapid increase in membrane potential and conductance (Fig. 3A), thus effectively antagonizing the pH-induced changes. The pH of the bathing solution did not change upon addition of up to 30 mM sodium salicylate, indicating that salicylate's antagonism was not the result of a buffering action. Furthermore, at least 98% of the salicylate was in the ionized form in the lowest pH solution, indicating that the effects seen were due to the salicylate anion. Salicylate delayed, but was not able to prevent the progressive deterioration of the cell caused by prolonged pH exposure. Washing with salicylate-free medium at pH 7.8 resulted in an initial depolarization to the same level as attained during exposure to the medium of pH 5.8, followed by a gradual return of both membrane potential and resistance to control values (Fig. 3A). The character of the membrane potential and resistance changes during wash-out suggests that the effects of salicylate were more easily reversed than are those of a decrease in pH.

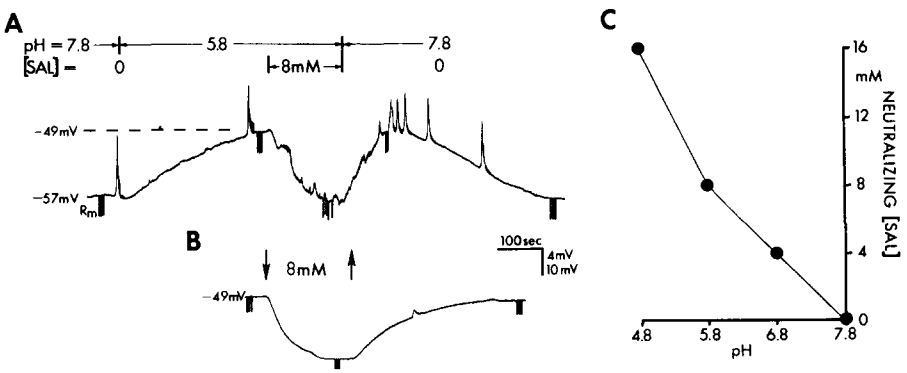

Fig. 3. A. Salicylate antagonizes the membrane depolarization produced by a medium of low pH. The results illustrated are from cell G-L and are representative of all cells studied. The input resistance of the cell was obtained at a resting membrane potential of –57 mV by passing 3-nA pulses, 800 ms long (R_m) through a second intracellular electrode. Pertusion of the 5-ml chamber with 50 ml of medium at pH 5.8 caused an 8 mV depolarization to –49 mV with little change in R_m. Application of 8 mM salicylate under these conditions led to an 8 mV hyperpolarization and 15% decrease in resistance. Washing with salicylate-free medium at pH 7.8 resulted in a re-depolarization followed by a return to control values. Calibration: 4 mV, 100 s. B. Application of 8 mM salicylate to the same cell artificially depolarized to –49 mV at pH 7.8 caused a 16 mV hyperpolarization with a 50% decrease in resistance. Calibration: 10 mV, 100 s. C. The average concentration of salicylate needed to restore membrane potential to control level is plotted as a function of pH of the bathing medium.

The decrease in pH also antagonized the effect of salicylate on membrane conductance. When a cell was artificially depolarized to a membrane potential of −49 mV, application of 8 mM salicylate produced a 16 mV hyperpolarization and a 50% decrease in input resistance (Fig. 3B). However, when the cell was depolarized to −49 mV by exposure to medium of pH 5.8, application of 8 mM salicylate produced only an 8 mV hyperpolarization and a 15% decrease in resistance (Fig. 3A). Proportionally higher concentrations of salicylate were required to antagonize the membrane depolarizations produced by greater decreases in pH (Fig. 3C).

In order to elucidate the ionic mechanisms underlying salicylate's antagonism of the pH-induced depolarization, we changed either $[K^+]_o$, $[Na^+]_o$ or $[Cl^-]_o$ in the presence of salicylate under control conditions (pH 7.8) and at lower pH values. At pH 7.8 salicylate caused an increase in the slope of the curve of membrane potential as a function of $[K^+]_o$ (Fig. 2), as we have previously reported[4]. The addition of salicylate to medium of pH 5.8 led to a restoration of the normal slope of the curve over the 1–60 mM $[K^+]_o$ range (Fig. 2). Thus, the presence of salicylate led to a recovery of the cell's sensitivity to K^+. It is also apparent that the low pH medium antagonized the effect of salicylate since the curve in salicylate at pH 5.8 is interposed between the normal curve and the curve in salicylate at pH 7.8.

Salicylate attenuated the 10–15 mV depolarizations observed upon reducing $[Cl^-]_o$ at pH 5.8, indicating that salicylate can reduce the cell's permeability to Cl^- in the presence of a decrease in external pH. The 1–2 mV hyperpolarization of the membrane potential observed upon decreasing external Na^+ to 10 mM was not affected by the presence of salicylate in acid medium.

The results demonstrate that a decrease in pH caused a decrease in membrane potential and conductance in these molluscan neurons primarily by decreasing their relative K^+ conductance although a concomitant increase in Cl^- conductance could not be excluded. These events would act to bring the membrane potential nearer to firing threshold (and in fact, an increase in the firing frequency of vertebrate afferent fibers in response to a decrease in pH has been reported[6]). Salicylate antagonized these effects by increasing K^+ conductance and decreasing Cl^- conductance. This antagonism increased the membrane potential and conductance, thus decreasing the probability of reaching firing threshold.

Since it is known that a decrease in local pH can evoke painful responses in both animals and humans[7–18] and that certain painful pathologic conditions (which respond to salicylate) are associated with local decreases in pH (*e.g.* the decrease in the pH of the synovial fluid in rheumatoid arthritis[16–18]), it is possible that part of salicylate's analgesic ability derives from its antagonism of pH-dependent pain phenomena, through a mechanism similar to that observed in this system.

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