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ISOPRENALINE- AND NORADRENALINE-INDUCED HYPERPOLARIZATION OF GUINEA-PIG LIVER CELLS

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1 Effects of pretreatment with isoprenaline (Isop) or noradrenaline (NA) and various ionic environments on the NA-induced or Isop-induced hyperpolarization of guinea-pig liver cells were investigated by means of a microelectrode technique.

2 NA (5.9×10^{-6} M) decreased the membrane resistance, and hyperpolarized the membrane with or without generation of an initial transient small depolarization. The NA-induced initial depolarization was not dependent on the membrane potential and was increased by Isop (4.0×10^{-6} M) or glucagon (10^{-7} M).

3 In Ca-free solution, the NA-induced hyperpolarization became transient and a continuous depolarization followed in the presence of NA. Repetitive application of NA resulted in a complete disappearance of the NA-induced hyperpolarization and was replaced by a slowly developing depolarization with or without generation of the initial transient depolarization. In excess $[Ca]_o$, the NA or Isop-induced hyperpolarization was increased.

4 Both Isop and glucagon hyperpolarized the membrane and decreased the membrane resistance, to various degrees. Repetitive application of Isop or glucagon resulted in the disappearance of both Isop and glucagon-induced hyperpolarizations. Pretreatment with NA not only resulted in a recovery of both Isop and glucagon-induced hyperpolarizations, but also extensively enhanced the hyperpolarization.

5 After pretreatment with Isop, the NA-induced hyperpolarization was decreased in amplitude and duration and was followed by a slowly developing depolarization. After repetitive application of Isop, NA produced only depolarization of the membrane, and in these conditions, Isop, glucagon or ATP also depolarized the membrane. These depolarizations were reversed to hyperpolarizations by pretreatment with excess $[Ca]_o$.

6 After treatment with Na-deficient solution, NA depolarized the membrane and decreased the membrane resistance. Excess $[Ca]_o$ restored the NA-induced membrane response from one of depolarization to one of hyperpolarization.

7 In the presence of tetraethylammonium 10mM, the NA-induced hyperpolarization became transient or ceased and depolarization occurred with a reduction in the membrane resistance.

8 It is postulated that both NA and Isop increase the free $[Ca]_i$ by releasing bound Ca from storage sites and consequently an increase in K conductance follows. NA but not Isop promotes Ca-influx which replenishes the storage site. In Ca-depleted conditions, NA does not elevate the free $[Ca]_i$ to a threshold concentration required for hyperpolarization, probably because NA induces a small release of Ca from storage sites.

Introduction

There have been several reports that catecholamines induce changes in membrane potentials in liver cells. In the isolated liver of the mouse, adrenaline and isoprenaline (Isop) produced hyperpolarization (Petersen, 1974; Graf & Petersen, 1978). Application of noradrenaline (NA) and Isop to guinea-pig liver slices (Haylett & Jenkinson, 1972; Jenkinson & Koller, 1977) or of Isop to the perfused rat liver (Somlyo, Somlyo & Friedmann, 1971) evoked a hyperpolarization, whereas in the perfused dog liver, adrenaline caused depolarization and Isop produced hyperpolarization (Lambotte, 1973). Recently, Egashira (1980a) showed that application of NA (10^{-5} M) caused a biphasic response in guinea-pig liver cells, i.e. an initial depolarization followed by hyperpolarization. This result differs from the findings of Haylett & Jenkinson (1972) and the discrepancy was attributed to differences in the membrane potential, as only the hyperpolarizing component appeared when the membrane potential was lower than −45 mV, and

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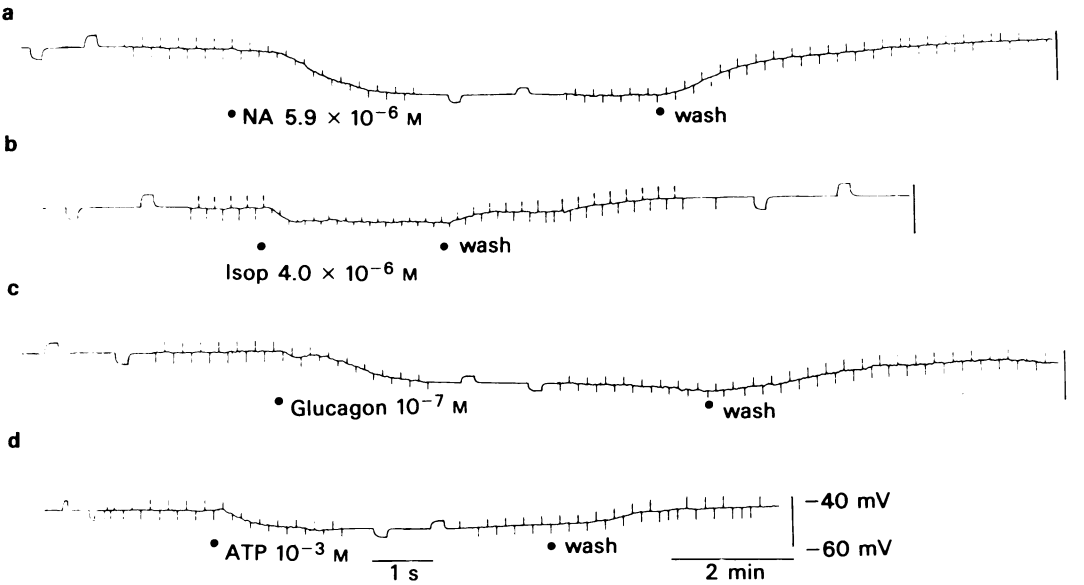

Figure 1 Effects of noradrenaline (NA), isoprenaline (Isop), glucagon and adenosine triphosphate (ATP) on the membrane potential and electrotonic potential of guinea-pig liver slice. (a) Application of NA 5.9 × 10^{-6} M; (b) application of Isop 4.0 × 10^{-6} M; (c) application of glucagon 10^{-7} M; (d) application of ATP 10^{-3} M. (●) Denotes application and removal of each drug. Two different speeds are shown. Stimulus intensity was 0.3 V/cm and duration was 200 ms.

the mean membrane potential reported by Haylett & Jenkinson (1972) was −36.2 mV.

Jenkinson & Koller (1977) found that Isop normally caused only a small and inconsistent increase in the membrane potential in guinea-pig liver cells, but after treatment with a selective α-adrenoceptor agonist, the response to Isop was considerably enhanced. Putney (1979) reviewed the stimulus-permeability coupling in liver cells and suggested that both NA and Isop may modulate membrane permeability, through intracellular Ca release from the same stores. Meanwhile, Egashira (1980b) observed that in Ca-free solution, the NA-induced hyperpolarization was divided into two phases i.e. an early and a late phase. The former was more resistant to the disappearance of the NA-induced hyperpolarization by removal of $[Ca]_o$. He suggested that the early phase of the NA-induced hyperpolarization is probably due to a release of the bound Ca and the late phase to Ca-influx from the external solution.

The object of the present experiments was to investigate further the nature of the initial depolarization (Egashira, 1980a) and to determine the effects of Isop or glucagon on NA-induced hyperpolarization.

Methods

Guinea-pigs of either sex, weighing 300 to 350 g, were stunned and bled. The left lobe of the liver was quickly isolated and a tissue slice (1.5 × 1.5 × 5 mm) was prepared from the edge of the lobe. The slice was mounted in a perspex bath (2 ml in capacity) superfused with Krebs solution bubbled with 3% CO_2 and 97% O_2, and maintained at 36 °C. The flow rate was kept constant at 2–3 ml/min and pH was 7.2–7.3. Modified Krebs solution (Bülbring, 1955) which served as the normal solution was of the following composition (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, $H_2PO_4^-$ 1.2, HCO_3^- 15.5 and glucose 11.5.

Na-deficient solution was prepared by replacing NaCl with equivalent amounts of Tris (Tris-(hydroxymethl)-aminomethane) Cl, choline chloride with 0.1 μg/ml atropine, sucrose of LiCl. In some experiments, KCl and KH_2PO_4 was replaced with equimolar RbCl. To prepare Ca^{2+}-free solution, $CaCl_2$ was omitted from the solution, and 0.5 mM EGTA was added. $MgCl_2$ 2.4 mM was added to restore the membrane potential to the Ca-free or Ca-deficient solution (see results).

An extracellular polarization method was used to produce the electrotonic potential in the hepatocyte (Egashira, 1980a). After incubating the preparation for more than 2 h, a conventional glass microelectrode filled with 3 M KCl was inserted into the liver cells through the liver capsule, with the aid of a binocular microscope. The resistance of the microelectrode ranged from 40 to 60 MΩ.

The following drugs were used at concentrations

(M) described in the results: (±)-noradrenaline HCl (Sankyo Pharm.), (−)-isoprenaline HCl (Nikken Chem.), atropine sulphate (Tanabe Pharm.), adenosine triphosphate sodium salt (Kowa), glucagon (Merck), tetraethylammonium chloride (Tokyo Kasei) ethylene glycol bis-(β aminoethyl ether)-*N*,*N*′-tetraacetic acid (EGTA: Wako).

The solutions were freshly prepared for each experiment. An interval of over 25 min was allowed between each drug application to avoid tachyphylaxis.

Results

Effects of noradrenaline, isoprenaline, glucagon and ATP on liver cell

The mean membrane potential of guinea-pig liver cells was −46.5 ± 5.4 mV s.d. (50 preparations, n= 280). NA (5.9×10^{-6} M) hyperpolarized the membrane to various degrees with or without generation of an initial small transient depolarization (initial depolarization). The maximum depolarization was 4 mV and the hyperpolarization ranged between 10 and 30 mV. The amplitude of the initial depolarization induced by NA was increased by pretreatment with Isop (4.0×10^{-6} M) or glucagon (10^{-7} M) but not with ATP (10^{-3} M), and was dependent on the membrane potential level. In two thirds of the specimens, NA produced the initial depolarization, and also in most cases after pretreatment with Isop or glucagon. In some specimens, a slowly developing depolarization continued for 5 to 10 min after removal of NA from the solution.

Both Isop (4.0×10^{-6} M) and glucagon (10^{-7} M) hyperpolarized the membrane to various degrees (up to 5 mV) but an initial depolarization was not observed. By repetitive or prolonged application of Isop or glucagon, the hyperpolarization induced by these agents became small and finally disappeared. ATP (10^{-3} M) produced a transient hyperpolarization, despite continuous application, as has been reported by Jenkinson & Koller (1977), but repeated application of ATP did not affect the ATP-induced hyperpolarization, when the interval of application was maintained to avoid development of tachyphylaxis.

Figure 1 shows the effects of NA (5.9×10^{-6} M), Isop (4.0×10^{-6} M), glucagon (10^{-7} M) and ATP (10^{-3} M) on the membrane potential and membrane resistance. Inward and outward current pulses (200 ms duration) were successively applied before, during and after treatment with these agents. NA hyperpolarized the membrane and decreased the amplitude of the electrotonic potential simultaneously (Figure 1a). Similarly, Isop, glucagon and ATP hyperpolarized the membrane and reduced the amplitude of the electrotonic potential.

To investigate further the effects of catecholamines

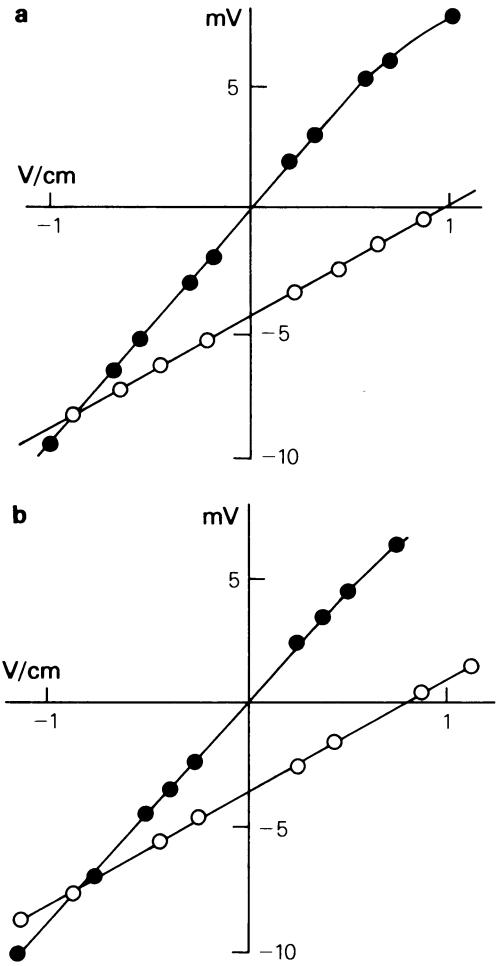

Figure 2 Effects of noradrenaline (NA 5.9×10^{-7} M) and isoprenaline (Isop 4.0×10^{-6} M) on the current-voltage relationship. (a) Current-voltage relationship observed before and during application of NA: (●) control; (○) noradrenaline. (b) Current-voltage relationship obtained before and during application of Isop: (●) control; (○) isoprenaline. Relative values of current intensity were measured by recording the voltage gradient in the solution with two silver electrodes, 4 mm apart, in the chamber.

on the membrane resistance, the current-voltage relationship was observed before and during application of these compounds. The microelectrode was inserted into the cell at distances of about 0.1 mm from the stimulating electrode in Figure 2a and b. Outward current pulses demonstrated the rectifying property of the membrane.

Application of NA (5.9×10^{-7} M) or Isop (4.0×10^{-6} M) hyperpolarized the membrane about 4 mV and the current-voltage relationships, measured by

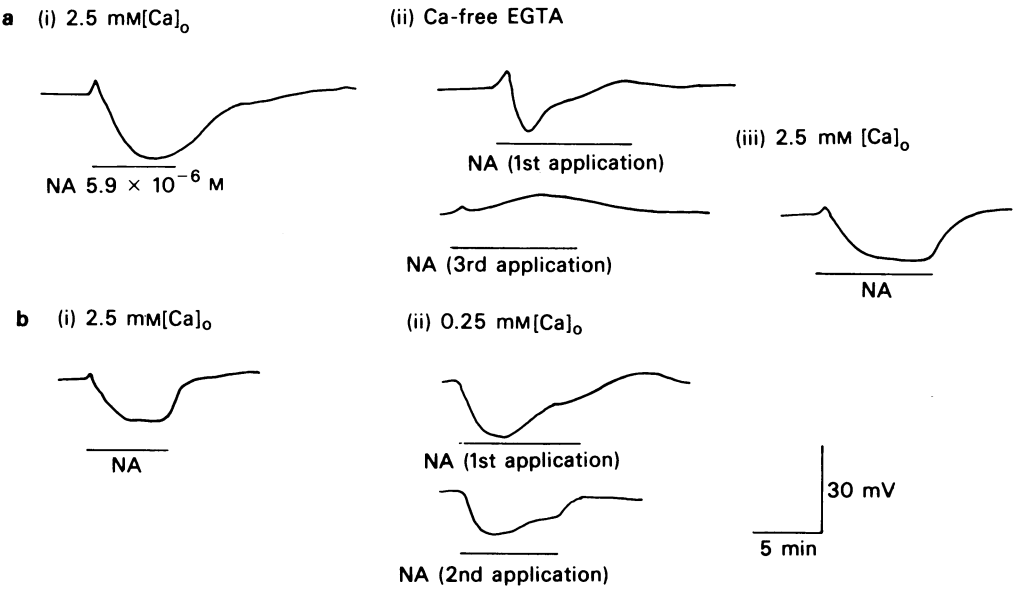

Figure 3 Effects of Ca-free (a) and Ca-deficient (b) environments on the hyperpolarization induced by noradrenaline (NA 5.9×10^{-6} M). (a)(i) Control; (ii) first and third application of NA in Ca-free solution, (iii) after control. (b)(i) Control; (ii) first and second application in 0.25 mM $[Ca]_o$.

application of inward and outward pulses, were consistently steeper in the controls than during application of catecholamine. When the amplitude of hyperpolarization induced by NA was much the same as that induced by Isop, the reduction in the membrane resistance induced by NA was also similar to that observed with Isop. This indicates that Isop increases K-conductance, as increase in K-conductance induced by NA has been verified by Haylett & Jenkinson (1972) and Egashira (1980a).

Effects of $[Ca]_o$ and noradrenaline on the hyperpolarization induced by noradrenaline or isoprenaline

Figure 3 shows effects of Ca-free (a) or Ca-deficient (b) solutions on the NA-induced hyperpolarization (5.9×10^{-6} M). To prevent marked depolarization of the membrane in Ca-deficient or Ca-free solution, 2.4 mM $[Mg]_o$, which was confirmed to have no effect on the membrane potential, was added. In Ca-free solution, the membrane was depolarized about 7 mV, and the NA-induced hyperpolarization appeared transiently and a depolarization developed slowly (late depolarization) during continuous application of NA (Figure 3a(ii)). This late depolarization reached the maximum value in about 10 min then declined gradually to the control level, in the presence of NA (20–30 min) in Ca-free solution. The maximum hyperpolarization decreased with the successive application of NA in Ca-free solution and with 3 or 4 applications, the NA-induced hyperpolarization disappeared and only a depolarization was apparent (Figure 3a(ii)). In Ca-free solution, the Isop-induced hyperpolarization was slightly decreased in amplitude but did not disappear on the first application.

The maximum amplitude of the NA-induced hyperpolarization (5.9×10^{-6} M) in 0.25 mM $[Ca]_o$ was much the same as that observed in the control solution (Figure 3b(ii)), in which the amplitude of the NA-induced hyperpolarization was sustained in the presence of NA. However, in 0.25 mM $[Ca]_o$ the NA-induced hyperpolarization declined to a certain sustained level (Figure 3b(ii)). By the second application of NA in 0.25 mM $[Ca]_o$, the maximum hyperpolarization was smaller than the first one in 0.25 mM $[Ca]_o$ and in the control; however, the sustained hyperpolarization level with the second application of NA was much the same as that observed with the first one (Figure 3b(ii)).

These phenomena suggest that the NA-induced hyperpolarization is divided into two phases, an early and a late phase. The late phase of the response was $[Ca]_o$-dependent but the early phase was not and depended on the previous application of NA rather than on the present application in Ca-free or deficient solution.

Figure 4a(i) and a(ii) show effects of pretreatment with NA (5.9×10^{-6} M) (i, 5 min; ii 10 min) on the Isop-induced hyperpolarization (4.0×10^{-6} M). After

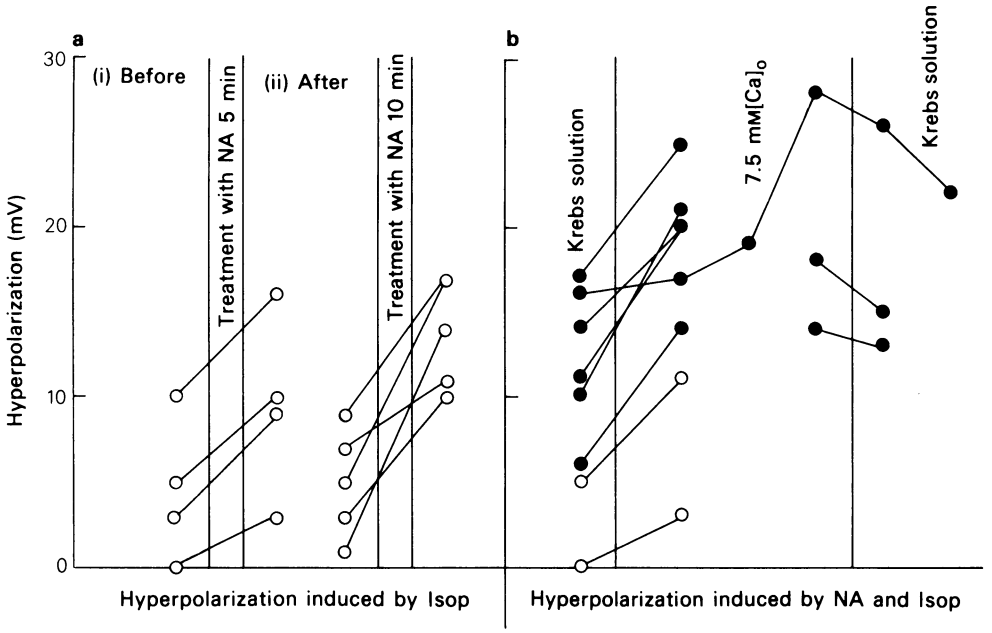

Figure 4 Effects of noradrenaline (NA 5.9×10^{-6} M) and isoprenaline (Isop 4.0×10^{-6} M) on the hyperpolarization induced by Isop or NA. (a) Effect of pretreatment with NA on the hyperpolarization induced by Isop: (i) treatment with NA for 5 min; (ii) treatment with NA for 10 min. (b) Effects of excess $[Ca]_o$ (7.5 mM) on the hyperpolarization induced by NA or Isop: (○) Isop: (●) NA. Each bar indicates the same cell: at left, before treatment; at right, after treatment.

pretreatment with NA, the Isop-induced hyperpolarization increased consistently. Figure 4b shows effects of excess $[Ca]_o$ on the NA-induced hyperpolarization. In 17.7 mM $[Ca]_o$, the membrane potential was much the same as that observed in the control solution. The maximum value of the hyperpolarization was increased in 17.7 mM $[Ca]_o$ and the hyperpolarization declined slowly to the NA-induced sustained hyperpolarization level seen in the control solution. The maximum amplitude of the hyperpolarization induced by the second application of NA in excess $[Ca]_o$ was larger than that induced by the first application in excess $[Ca]_o$. When the cell was rinsed again with the control solution, the first application of NA or Isop produced a larger hyperpolarization than was seen in the control before replacement with excess $[Ca]_o$.

Effects of isoprenaline on the noradrenaline-induced hyperpolarization

Figure 5a(i) and a(ii), (c) and (d) show effects of Isop (4.0×10^{-6} M) ((i), 5 min; (ii) 10 min) on the hyperpolarization induced by NA (5.9×10^{-6} M) or Isop (4.0×10^{-6} M). Pretreatment with Isop (Figure 5a(i) 5 min; a(ii) 10 min) decreased the NA-induced hyperpolarization. Furthermore, the hyperpolarization induced by the second application of Isop became consistently smaller than that induced by the first application of Isop (Figure 5c). By pretreatment with glucagon (10^{-7} M), the NA-induced hyperpolarization was also decreased. These effects of both Isop and glucagon seem similar to those induced by NA in Ca-free solution, i.e. the second application of NA produced only a slight hyperpolarization, compared with that produced by the first application of NA.

Effects of NA or Isop in Ca-free solution on the following hyperpolarization induced by each substance in the control solution were also observed. The NA-induced hyperpolarization (5.9×10^{-6} M) was not affected by pretreatment with NA (5.9×10^{-6} M) for about 5 min in Ca-free solution (Figure 5b). On the other hand, pretreatment with Isop (4.0×10^{-6} M) for about 5 min in Ca-free solution (Figure 5d), greatly reduced the Isop-induced hyperpolarization in the control solution.

Figure 6 shows effects of Isop on the NA-induced hyperpolarization (5.9×10^{-6} M). After treatment with Isop (4.0×10^{-6} M), the NA-induced hyperpolarization became small in amplitude and transient in duration and the late depolarization appeared during continuous application of NA (Figure 6b(ii) and (iii)). By further repeated pretreatment with Isop, NA produced only a depolarization (Figure 6a(ii) and

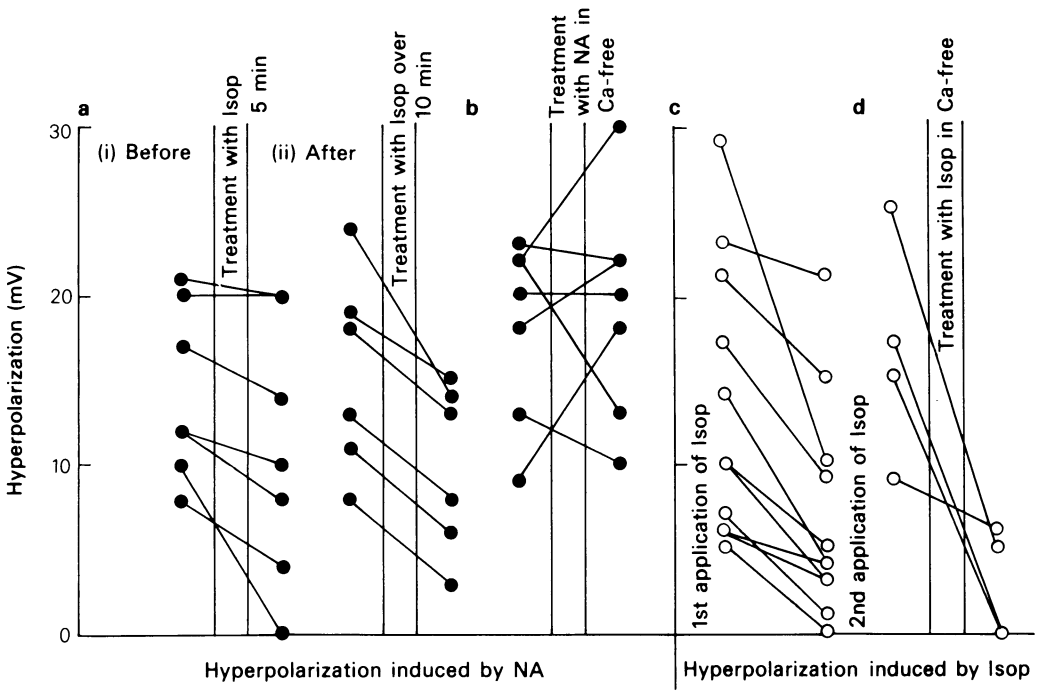

Figure 5 Effects of isoprenaline (Isop 4.0×10^{-6} M) on the hyperpolarization induced by noradrenaline (NA 5.9×10^{-6} M) or Isop. (a) Effect of pretreatment with Isop on the hyperpolarization induced by NA: (i) pretreatment with Isop 5 min; (ii) pretreatment with Isop over 10 min. (b) Effect of pretreatment with NA (5 min) in Ca-free solution on the hyperpolarization induced by NA. (c) Comparison between first application of Isop and second application in control solution. (d) Effect of pretreatment with Isop (5 min) in Ca-free solution on the hyperpolarization induced by Isop. Each bar indicate the same cell: at left, before treatment; at right, after treatment.

b(iv)). Under these conditions application of Isop, glucagon or ATP also depolarized the membrane (Figure 6b(iv)). In Figure 6b(iv), the initial depolarization was detected by pretreatment with Isop, and when the late depolarization became large in amplitude, the initial depolarization could not be differentiated from the late depolarization (Figure 6b(ii) and (iv)). Pretreatment with 7.5 mM $[Ca]_o$ for about 10 min produced a recovery of usual responses to these agents in the control solution i.e. the membrane was again hyperpolarized by application of these agents.

Initial depolarization

As shown in Figure 7, when the NA-induced hyperpolarization disappeared in Ca-free solution or after treatment with Isop (4.0×10^{-6} M), the entire shape of the initial depolarization could be traced. Both 5.9×10^{-6} M and 5.9×10^{-5} M NA produced much the same amplitude of initial depolarization (Figure 7a(ii), b(ii)). However, the late depolarization induced by NA 5.9×10^{-5} M was larger than that induced by NA 5.9×10^{-6} M. NA (5.9×10^{-6} M) produced the initial depolarization, then the late depolarization. The amplitude of the late depolarization induced by NA 5.9×10^{-6} M was larger than that of the initial depolarization.

When 5.9×10^{-5} M NA was applied in the presence of 5.9×10^{-6} M NA, the initial depolarization consistently appeared with the same amplitude as that produced by 5.9×10^{-5} M NA alone. This occurred during generation of both the 5.9×10^{-6} M NA-induced hyperpolarization in the control solution and the 5.9×10^{-6} M NA-induced late depolarization in Ca-free solution, or after treatment with Isop (Figure 7a(ii), b(ii)).

Effects of deficient $[NA]_o$ and tetraethylammonium on the noradrenaline-induced hyperpolarization

Figure 8 shows the effects of a 15 min application of Na-deficient solution on NA-induced hyperpolarization. In Na-deficient solution (15.4 mM) with Na substituted for by Tris Cl or choline Cl, the membrane was depolarized about 5 mV. The NA-induced hyperpolarization was completely suppressed, and the NA-

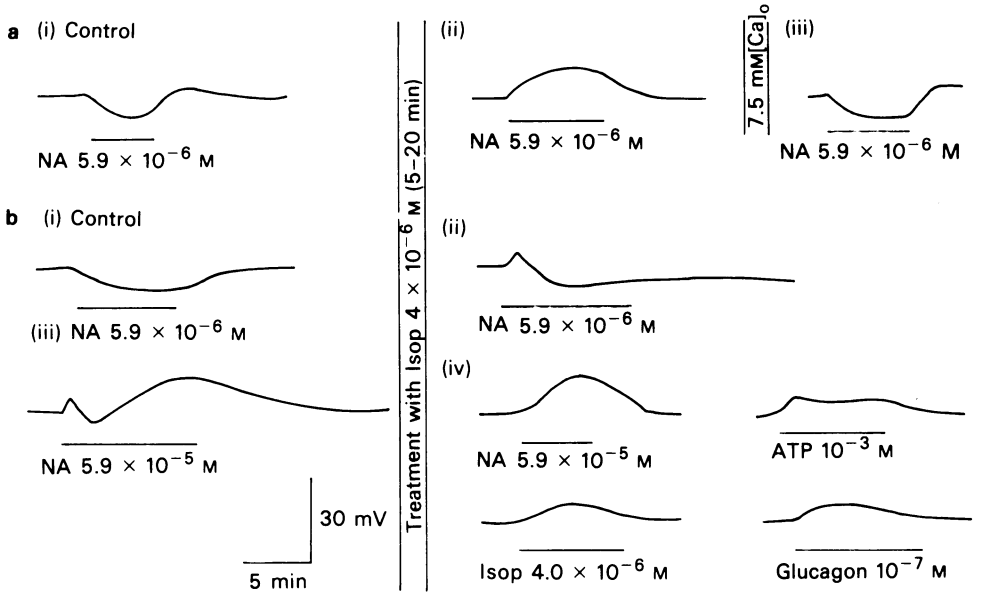

Figure 6 Effect of pretreatment with isoprenaline (Isop 4.0 × 10 $^{-6}$ M, 5 min) on the hyperpolarization induced by noradrenaline (NA 5.9 × 10 $^{-6}$ M), isoprenaline (Isop 4.0 × 10 $^{-6}$ M), adenosine triphosphate (ATP 10 $^{-3}$ M) and glucagon (10 $^{-7}$ M). (a) Effect of pretreatment with Isop for 20 min on the hyperpolarization induced by NA and effect of pretreatment with excess (7.5 mM) $[Ca]_o$ on the recovery to the hyperpolarizing response to NA: (i) control; (ii) after treatment with Isop; (iii) after treatment with 7.5 mM $[Ca]_o$. (b) Effect of pretreatment with Isop on the hyperpolarization induced by each drug: (i) control; (ii) after treatment with Isop for 5 min; (iii) application of further increase in the concentration of NA (5.9 × 10 $^{-5}$ M); (iv) after treatment with Isop for 10 min. (a)(i)–(iii) and (b)(i)–(iv) were recorded from the same cell.

induced initial and late depolarizations were smaller than those induced by pretreatment with Isop (Figure 8a(ii)). When the solution was again replaced with the control solution, the first application of NA produced slightly larger initial and late depolarizations than were seen in the $[Na]_o$ deficient solution without any hyperpolarization (Figure 8a(iii)). Repetitive application of NA in the control solution slowly restored generation of the NA-induced hyperpolarization (Figure 8a(iii)). By pretreatment with 7.5 mM $[Ca]_o$, the membrane again generated the NA-induced hyperpolarization of full amplitude (Figure 8a(iv)). In 68.7 mM $[Na]_o$, suppression of the NA-induced hyperpolarization was still observed, but not with a dose of 110.0 mM $[Na]_o$. When NaCl in the control solution was replaced with LiCl (15.4 mM $[Na]_o$), the membrane depolarized to a greater extent than was seen in the case of choline Cl or Tris Cl substitution, and the reduced membrane potential was not restored after wash out of the LiCl. With the Li substitution, the NA-induced hyperpolarization could no longer be observed and the depolarization was more suppressed than with Tris or choline substitution. When NaCl was replaced with sucrose (15.4 mM $[Na]_o$), the membrane was slightly depolarized and the NA-induced hyperpolarization also decreased, but not to zero. When KCl and KH_2PO_4 were replaced by RbCl, (0 mM $[K]_o$), the response to NA was similar to that in the control.

Figure 8b shows effects of TEA on the NA-induced hyperpolarization. In the presence of TEA 5 × 10 $^{-3}$ M, NA produced a smaller amplitude of hyperpolarization than was seen in the control solution (Figure 8b(ii)). In the presence of TEA 10 $^{-2}$ M, the NA-induced hyperpolarization was suppressed and only the depolarization appeared (Figure 8b(iii)). To investigate the nature of the NA-induced depolarization after disappearance of the hyperpolarization, the membrane resistance was measured (Figure 9 a–c). Inward and outward current pulses (200 ms duration) were alternately applied before, during and after treatment with NA. In the control solution, NA (5.9 × 10 $^{-6}$ M) hyperpolarized the membrane and reduced the amplitude of electrotonic potentials (a). In 15.4 mM $[Na]_o$, the NA-induced hyperpolarization disappeared and a slight depolarization appeared with a

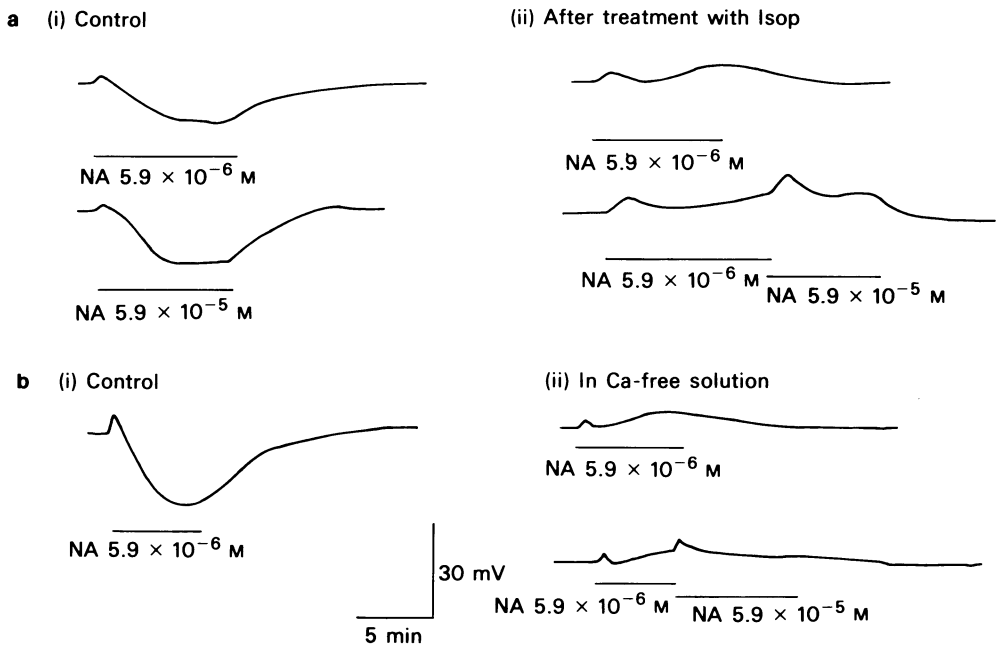

Figure 7 Effects of further increase in the concentration of noradrenaline (NA 5.9×10^{-5} M) on the NA (5.9×10^{-6} M)-induced depolarization after treatment with isoprenaline (Isop 4.0×10^{-6} M 10 min) (a) or in Ca-free solution (b). (a)(i) Control; (ii) after treatment with Isop. (b)(i) Control; (ii) in Ca-free solution.

reduction in the amplitude of electrotonic potentials (b). When this solution was replaced with the control one, the first application of NA produced a larger depolarization than was observed in 15.4 mM $[Na]_o$, yet the reduction in the membrane resistance was much the same as that seen in 15.4 mM $[Na]_o$ (Figure 9c). In the presence of 10 mM TEA, the NA-induced hyperpolarization was decreased and there was a reduction in the membrane resistance (Figure 9d).

Discussion

Initial depolarization

Egashira (1980a) found that in guinea-pig liver cells, the application of NA (10^{-5} M) produced an initial depolarization followed by a hyperpolarization, and that the response to NA depended on the membrane potential, i.e., the depolarizing component of the biphasic response was larger with a higher membrane potential, while the hyperpolarizing component of the biphasic response was larger with a lower membrane potential. He also reported that when the membrane potential was lower than −45 mV, the depolarizing component was not observed.

In the present experiments, the mean membrane potential was −46.5 mV, and the initial depolarization was observed in two thirds of all cells used. By pretreatment with Isop or glucagon, the initial depolarization was increased in amplitude and was generated from almost all preparations without any apparent change in the membrane potential. Furthermore, the amplitude of the initial depolarization was not dependent on the membrane potential, i.e. as shown in Figure 7a(ii) and b(ii), the NA-induced initial depolarization showed much the same amplitude during either depolarization or hyperpolarization. The initial depolarization was more sensitive to Isop or glucagon than to changes in the ionic environments.

Hyperpolarization and the role of Ca

Haylett (1976) observed a concomitant increase in the effluxes of Ca and K from guinea-pig liver slices following application of α-adrenoceptor agonists, and suggested that an increase in the free $[Ca]_i$ may be responsible for increases in K-conductance. Weiss & Putney (1978) observed that NA and ATP caused a transient release of K from guinea-pig liver slices and concluded that this increase in K permeability is Ca-

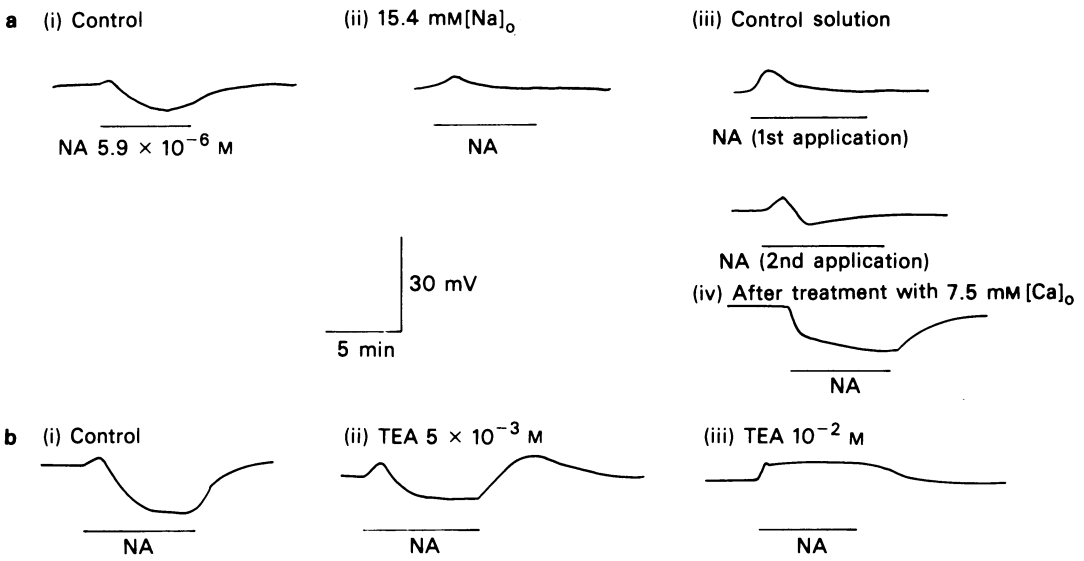

Figure 8 Effects of deficient $[Na]_o$ environment (15.4 mM $[Na]_o$) (a) and tetraethylammonium (TEA) (b) on the hyperpolarization induced by noradrenaline (NA 5.9×10^{-6} M). (a)(i) Control; (ii) in 15.4 mM $[Na]_o$ replaced with choline chloride; (iii) first and second applications of NA in control solution after return from 15.4 mM $[Na]_o$; (iv) after treatment with 7.5 mM $[Ca]_o$. (b)(i) Control; (ii) in the presence of TEA 5×10^{-3} M; (iii) in the presence of TEA 10^{-2} M.

mediated and that the increase in free $[Ca]_i$ is not mediated by Ca-influx but rather by a transient release of bound Ca, while Ca influx is required to obtain a subsequent response. Egashira (1980b) observed that in Ca-free solution, the NA-induced hyperpolarization was divided into two phases i.e. an early and a late phase. The former was more resistant to abolition of NA-induced hyperpolarization by the removal of external Ca. He suggested that the NA-induced hyperpolarization was due to an increase in the K conductance as a result of an increase in the intracellular Ca concentration, and therefore that the early phase of the NA-induced hyperpolarization was due to a release of bound Ca and the late phase to Ca-influx from the external solution.

In the present experiments, the NA-induced hyperpolarization was also divided into an early and a late phase. Because the early phase was observed in Ca-free solution with the first application of NA, this may be due to a release of bound Ca and the late phase to Ca-influx from the external solution, as suggested by Egashira (1980b). This early hyperpolarizing phase in Ca-free solution disappeared with repeated application of NA. This phenomenon may be due to depletion of Ca from storage sites, since Ca influx does not appear in Ca-free solution and therefore no replenishment of the bound Ca occurs.

α and β actions on storage sites

β-Agonists do not usually stimulate K efflux from guinea-pig liver cells but will do so if the tissue is pretreated with an α-agonist. When α- and β-agonists were added in series while monitoring the efflux of ^{45}Ca, the β-agonist induced a considerable stimulation of ^{45}Ca efflux. When the β-agonist was added first, no stimulation of ^{45}Ca release was observed (Jenkinson, Haylett & Koller, 1978). Putney (1979) discussed these phenomena as follows; when α-agonists elevate free $[Ca]_i$, intracellular Ca storage sites may act to sequester the Ca, and these sites are the same as those discharged by β-agonists (or cyclic AMP). Pretreatment with α-agonist may produce much higher concentrations of bound Ca as compared to that occurring in untreated tissue. Therefore, Putney (1979) considered that under these conditions, the concentration of Ca exceeds the threshold required for K release by application of a β-agonist.

In the present experiments pretreatment with NA increased the Isop-induced hyperpolarization. In excess Ca solution, the second application of NA produced a larger hyperpolarization than the first. These findings are explainable if the increase in Ca-influx induced by NA results in a greater replenishment of Ca in the storage sites and thereby produces a

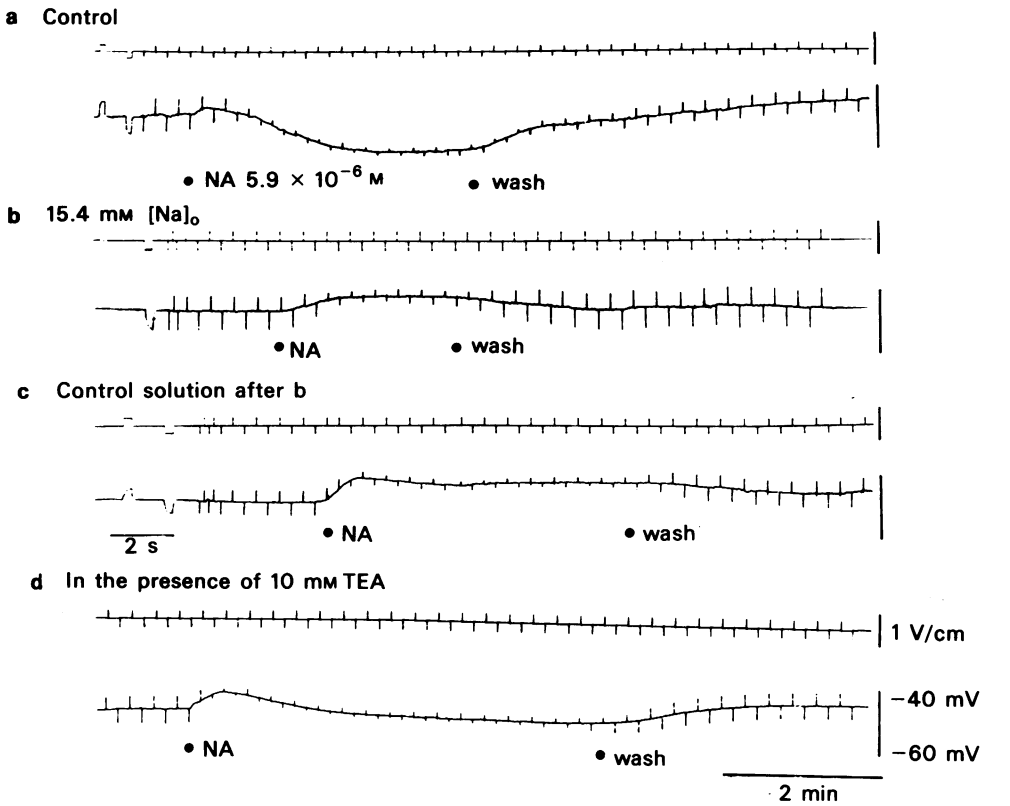

Figure 9 Effect of noradrenaline (NA 5.9 × 10^{-6} M) on the membrane potential and electrotonic potential in control (a), in 15.4 mM $[Na]_o$, replaced with choline chloride (b), in control solution after return from 15.4 mM $[Na]_o$ (c) and in the presence of 10 mM tetraethylammonium (TEA) (d). (●) Application and removal of NA. Two different speeds are shown. Stimulus duration is 200 ms.

greater release of Ca by subsequent application of agonists. Only conditions of excess Ca also replenish the Ca storage sites, since such conditions restored the NA-induced hyperpolarization from the NA-induced depolarization produced by pretreatment with Isop or deficient Na.

As Isop hyperpolarized the membrane in Ca-free solution, the release of the bound Ca plays a role. Repeated application of Isop but not NA in a Ca-free solution decreased the hyperpolarization evoked in the control solution. This indicates that Isop has a larger Ca-releasing action, and if Ca-influx were induced by Isop to much the same level as by NA, the Isop-induced hyperpolarization would be larger than the NA-induced one. However, the results were the reverse of this, indicating that Isop-induced Ca-influx is much smaller than NA-induced influx. As a consequence, as Isop releases Ca from storage sites, and does not replenish them, Isop depletes Ca-storage sites. Under these conditions, NA no longer produced the Ca-induced increase in the K-conductance, and this may be explained if the NA-induced release of little Ca from the depleted storage sites does not exceed the threshold concentration required for activation of K conductance.

Na-deficient solutions had much the same effect as treatment with Isop, i.e. there was a decrease or a cessation of the NA-induced hyperpolarization. After treatment with excess $[Ca]_o$, the NA-induced hyperpolarization was restored. Therefore, a state of Na-deficiency results in a decrease in stored Ca which is released by agonists.

Na-conductance

The NA-induced depolarization in deficient-Na solution was less than that produced by pretreatment with Isop, and also less than that produced in control solution after washing out the Na-deficient solution. Under these conditions, NA depolarized the mem-

brane and there was a decrease in membrane resistance. TEA decreases the K-conductance selectively in various tissues (Hagiwara & Saito 1959, Nakajima 1966; Ito, Kuriyama & Sakamoto, 1970). In the present experiments, by pretreatment with TEA 10 mM, the NA-induced hyperpolarization was replaced by a depolarization with reduction in the membrane resistance. These phenomena indicate that NA increases both K and Na conductances and that normally, NA increases the K conductance more than the Na-conductance of the membrane. It is still not clear whether an increase in Na-conductance is Ca-dependent or is activated directly by NA, because the NA-induced late depolarization requires rather a long time to disappear in Ca-free solution. If the increase in the Na-conductance is indeed Ca-dependent, the threshold concentration of Ca required to activate the Na-conductance will be lower than that required to activate K-conductance.

It is postulated that NA produces an elevation of the $[Ca]_i$ by releasing bound Ca from the storage sites thereby increasing K conductance and in addition, promoting Ca-influx and replenishing the storage sites with Ca. Isop releases Ca from storage sites but does not increase Ca-influx, thus deplenishing the storage sites. Na-deficient solutions also deplete bound Ca from the storage sites. These depletions of stored Ca suppress the Ca-induced increase in the NA-induced K-permeability, but not the increase in Na-permeability; thus the NA-induced response of hyperpolarization is replaced by one of depolarization.

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