Calcium and Action Potentials of Bullfrog Sympathetic Ganglion Cells

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ABSTRACT Bullfrog sympathetic ganglion cells were capable of producing action potentials (Ca spikes) in an isotonic (84 mM) $CaCl_2$ solution. The peak level of Ca spikes showed an approximately 30 mv increase with a 10-fold increase in the Ca concentration. Na as well as Ca ions were capable of acting as charge carriers during the production of action potentials in a solution containing relatively high Ca and relatively low Na ions. A decrease in the external Ca concentration depressed the maximum rate of rise at a fixed resting potential level, and increased the maximum rate of rise of the Na spikes at a high resting potential level at which Na inactivation was completely depressed. Compared to Na spikes, Ca spikes were less sensitive to TTX and procaine. Ganglion cells were also capable of producing action potentials (Sr spikes) in an isotonic $SrCl_2$ solution and prolonged action potentials in an isotonic $BaCl_2$ solution, but these cells were rendered inexcitable in an isotonic $MgCl_2$ solution. The peak level of the Sr spikes was dependent on the external Sr concentration and was insensitive to both TTX and procaine. Sr ions, like Ca ions, reduced Na inactivation during the resting state, and depressed the maximum rate of rise of the Na spikes at a high resting potential level. It was concluded that Ca (and Sr) ions exert dual actions on the membrane; namely, regulating the Na permeability and acting as charge carriers during the active state of the membrane.

INTRODUCTION

It has long been known that action potentials of certain kinds of excitable cells, e.g., arthropod muscle fibers (Fatt and Katz, 1953; Fatt and Ginsborg, 1958; Werman and Grundfest, 1961; Werman, McCann, and Grundfest, 1961), amphibian spinal ganglion cells (Tasaki, 1959; Nishi, Soeda, and Koketsu, 1965), or mammalian nerve fibers (Greengard and Straub, 1959), can be elicited in a solution in which the sodium (Na) ions are totally replaced by an alkali-earth cation, such as barium (Ba) or strontium (Sr). These action potentials are dependent on the concentration of divalent cations in the external solution, which suggests that the inward movement of the divalent cations across the cell membrane is responsible for the production of action potentials. It is also known that crustacean muscle fibers (Fatt and Katz, 1953; Fatt and

Ginsborg, 1958) or amphibian spinal ganglion cells (Koketsu, Cerf, and Nishi, 1959 *a*, *b*), when treated with quaternary ammonium ions, are capable of producing action potentials in a sucrose solution containing only $CaCl_2$. Since the peak level of the action potentials thus produced is dependent on the Ca concentration of the medium, the inward movement of Ca appears to be responsible for the production of the action potentials (Fatt and Katz, 1953; Fatt and Ginsborg, 1958). More recently, evidence that Ca can act as a charge carrier during the production of the action potential has been found with certain kinds of crustacean muscle fibers (Hagiwara, Chichibu, and Naka, 1964; Hagiwara and Naka, 1964; Abbott and Parnas, 1965) and molluscan giant nerve axons (Tasaki, Watanabe, and Singer, 1966) or cells (Gerasimov, Kostyuk, and Maiskii, 1964; Meves, 1967). These cells are capable of producing action potentials in an isotonic $CaCl_2$ solution, with the peak level of the action potential determined by the Ca concentration of the medium.

There is a strong possibility that Ca ions are acting as charge carriers during the production of the action potential in a solution containing Na ions. The Ca influx has been known to increase during the action potential of the squid giant axon, although the charge carried by Ca ions may be very small compared with that carried by Na ions (Flückiger and Keynes, 1955; Hodgkin and Keynes, 1957). Furthermore, evidence showing that Ca ions may be acting as charge carriers during the action potential of heart muscle fibers has been presented (Niedergerke and Orkand, 1966 *a*, *b*; Hagiwara and Nakajima, 1965, 1966; Reuter, 1967). The inward movement of Ca ions seems to be at least partially, also responsible for the production of the action potentials of mammalian smooth muscle fibers (Holman, 1958; Bülbring and Kuriyama, 1963; Brading and Tomita, 1968).

The present paper reports the observation that amphibian sympathetic ganglion cells are capable of producing action potentials in an isotonic $CaCl_2$ solution as well as in an isotonic $SrCl_2$ or $BaCl_2$ solution. Experimental evidence will be presented to show that Ca (and Sr) ions are able to control the Na conductance change while acting as charge carriers during the action potential. A preliminary account of the present study has appeared in *Nature* (Koketsu and Nishi, 1968).

METHODS

Isolated lumbar sympathetic ganglia of bullfrog (*Rana catesbeiana*) were used throughout. The method for recording the action potentials of ganglion cells, produced by stimulating currents directly applied through a recording intracellular microelectrode, was essentially similar to that described elsewhere (Nishi and Koketsu, 1960). A ganglion was continuously perfused with either Ringer solution or a test solution; the perfusate could be changed to a desired solution at any time during the experiment. All experiments were carried out at room temperature (22–23°C). The

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composition of the Ringer solution was as follows: 112 mM (millimoles per kilogram of water) NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, and 2 mM $NaHCO_3$. The solutions containing 84 mM of $CaCl_2$, $SrCl_2$, $BaCl_2$, or $MgCl_2$ and 2 mM KCL or $KHCO_3$ were considered to be approximately isotonic. To prepare a solution containing less than 84 mM alkali-earth cations, one of these solutions was mixed with an isotonic sucrose solution (224 mM sucrose and 2 mM $KHCO_3$) in an appropriate ratio. A solution which contained certain amounts of Na, Ca, or Sr ions was prepared by mixing an isotonic NaCl (112 mM NaCl), $CaCl_2$, $SrCl_2$, or sucrose solution in an appropriate ratio. For experiments in which the effects of Ca or Sr ions on the Na spikes were studied, a modified Ringer solution (87.7 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 2 mM $NaHCO_3$, and 49.6 mM sucrose), a high Ca Ringer solution (87.7 mM NaCl, 2 mM KCl, 18 mM $CaCl_2$, and 2 mM $NaHCO_3$), and a low Ca Ringer solution (87.7 mM NaCl, 2 mM KCl, 0.18 mM $CaCl_2$, 2 mM $NaHCO_3$, and 53.5 mM sucrose) were used. Tetrodotoxin (TTX) was obtained from Sankyo Co., Ltd., Japan. The pH of the solutions containing 2 mM $NaHCO_3$ and that of the unbuffered solutions ranged between 7.1 to 7.3 and 6.0 to 6.5, respectively.

Glass capillary microelectrodes (15-20 MΩ) filled with 3 M KCl were used throughout to record the potential changes. A calomel electrode (Beckman) was used as the indifferent electrode. The tip potential (cf. Adrian, 1956) of a microelectrode varied according to the solution used. In order to determine the actual potential level in a test solution, the difference between the potential level in Ringer and that in a test solution was measured by the microelectrode used for experiments; the difference was obtained by measuring the changes in the potential level when the perfusate was changed from Ringer to a test solution. The actual potential level in a test solution could thus be obtained by assuming that the junctional potential of the indifferent calomel electrode was constant and also that the difference between the tip potential of the microelectrode in Ringer solution and that in the intracellular fluid was negligible.

RESULTS

1. *Ca Spikes in Isotonic $CaCl_2$ Solution*

Bullfrog sympathetic ganglion cells were capable of producing action potentials (Ca spikes) in an isotonic $CaCl_2$ solution when they were directly stimulated by cathodal currents applied through an intracellular recording microelectrode. Since Ca spikes could be recorded even after the ganglion had been perfused continuously with an isotonic $CaCl_2$ solution for 2–7 hr, the possibility that Na ions, retained in the extracellular space, might be responsible for the production of the action potentials could be dismissed; the superficially located cells of the ganglion were rendered inexcitable within 10 min of perfusion with an isotonic sucrose solution. The value of the resting potential in an isotonic $CaCl_2$ solution was larger than that in Ringer solution; the former ranged from −80 to −110 mv (among 10 cells), whereas the latter ranged from −50 to −70 mv (among 20 cells), depending on individual cells. The effective resistance of the resting cell membrane in an isotonic $CaCl_2$ solution also was considerably larger than that in Ringer solution; the mean

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value of the former among 7 cells was 187 ± 15 MΩ (SE of mean), whereas that of the latter among 10 cells was 38.3 ± 1.6 MΩ (SE of mean).

Ca spikes were initiated when the membrane was depolarized to a certain

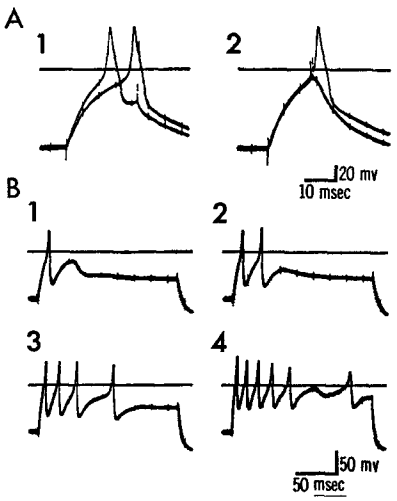

FIGURE 1. A, the action potentials (Ca spikes) of two different cells in an isotonic $CaCl_2$ solution; cathodal pulses of approximately 21 and 13 msec duration were used for records 1 and 2, respectively. B, repetitive responses (Ca spikes) of a cell in an isotonic $CaCl_2$ solution; the strength of cathodal pulses of approximately 170 msec duration was progressively increased from records 1 to record 4.

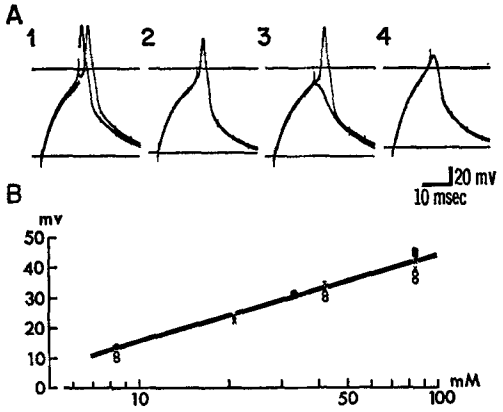

FIGURE 2. A, changes in the peak potential levels of Ca spikes of a single cell in solutions containing different amounts of Ca ions. The perfusate was changed from isotonic $CaCl_2$ (84 mM) solution (record 1) to solutions containing 33.6 mM (record 2), 84 mM (record 3), and 8.4 mM (record 4) Ca ions, successively; records 2, 3, and 4 were obtained approximately 3 min after perfusion with a solution. B, relationship between the peak potentials of Ca spikes and the concentrations of Ca ions in the external solution, constructed from data obtained from three different cells. Filled circles represent the data shown in A.

potential level (Fig. 1 A). The threshold for initiation of Ca spikes was much higher than that for Na spikes in Ringer solution; the threshold potential level for the Ca spikes ranged from −5 to −15 mv (among 10 cells), whereas that

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for the Na spikes ranged from −40 to −45 mv (among 20 cells). The peak potential of Ca spikes ranged from +40 to +55 mv, with the spike duration approximately 3–5 msec (among 10 cells). Repetitive Ca spikes were observed when a strong cathodal current was applied (Fig. 1 B). The maximum rate of rise of Ca spikes (10 cells) varied largely according to individual cells; its mean value (63 v/sec among 10 cells) was approximately 42 % of that of Na spikes (20 cells). The IS (initial segment) component, which was isolated from the Na spikes (Nishi and Koketsu, 1960), could not be isolated from the Ca spikes. This together with the fact that the threshold for Ca spikes was very high suggested that only part of the cell body membrane was capable of producing Ca spikes (Nishi and Koketsu, 1960).

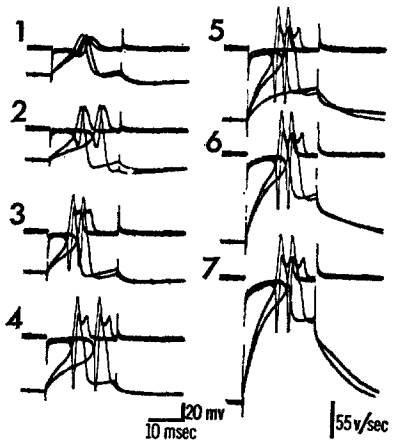

FIGURE 3. Effect of altering the membrane potential of a ganglion cell on the maximum rate of rise of Ca spikes in an isotonic $CaCl_2$ solution. The membrane potential was altered by applying conditioning cathodal (records 1–5) or anodal (record 7) currents; record 6 was obtained at resting potential level. The trace for the electrically differentiated potential change of the Ca spikes represents the zero potential level.

2. *Ca Spikes and External Ca Concentration*

The peak potential as well as the maximum rate of rise of the Ca spikes was dependent on the Ca concentration in the external solution; $CaCl_2$ in the isotonic $CaCl_2$ solution was replaced in part with equimolar amounts of sucrose (see Methods). The resting potential of cells dropped when the external Ca concentration was reduced, and the cells were rendered inexcitable when the external Ca concentration was less than 5 mM; the resting potential in a solution containing 5 mM $CaCl_2$ was approximately −60 to −80 mv. The relation between the peak potential of the Ca spikes and the logarithm of the external Ca concentration was an almost straight line; the decrement of the peak potential was about 30 mv with a one-tenth decrease in

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the Ca concentration (Fig. 2). The graph shown in Fig. 2 was constructed from data obtained from three different cells. In each cell, the Ca spikes were first recorded in an isotonic $CaCl_2$ solution, and then in a test solution containing certain amounts of Ca ions; recovery of the Ca spikes in the isotonic solution was confirmed.

3. *Ca Inactivation*

The maximum rate of rise, which is proportional to the inward current during the production of Ca spikes (cf. Narahashi, 1961, 1964; see also Hodgkin and Katz, 1949), was dependent on the resting potential level which could be altered by applying extrinsic cathodal or anodal currents through the record-

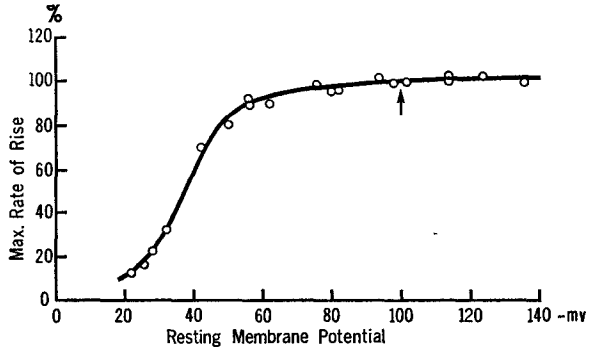

FIGURE 4. Inactivation curve of the Ca spike in an isotonic $CaCl_2$ solution. Abscissa, membrane potential at which Ca spike was produced. Ordinate, relative value of maximum rate of rise of the Ca spikes, which is scaled in percentage. The maximum rate of rise of the Ca spikes, produced at a membrane potential at which Ca inactivation was fully depressed, was taken to be 100%. Resting potential is shown by arrow. Duration of conditioning pulses was approximately 300 msec.

ing microelectrode. It decreased when the resting membrane was depolarized to a certain potential level by a conditioning cathodal current, whereas it did not show any appreciable change when the resting membrane was hyperpolarized by a conditioning anodal current (Fig. 3). This indicated that Ca inactivation, similar to Na inactivation, took place when the membrane was depolarized. The inactivation curve, which represents the relation between the maximum rate of rise of the Ca spikes and the membrane potential level, is shown in Fig. 4. It appeared that inactivation of the Ca-carrying system was almost completely depressed when the membrane potential was maintained at a level higher than approximately −80 mv in a solution containing more than 8.4 mM Ca.

4. *Effects of Ca on Na Spikes*

The effects of Ca ions in the external solution on the changes in the Na conductance during the action potential (Na spikes) were fully investigated with

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squid giant axon using the voltage-clamp method (Frankenhaeuser and Hodgkin, 1957). According to this experiment, decreasing the external Ca concentration increased the proportion of the Na-carrying system which was in an inactive or refractory condition at a constant membrane potential, and shifted the Na conductance membrane potential curves along the voltage axis so that a smaller depolarization was required to produce a given rise in Na

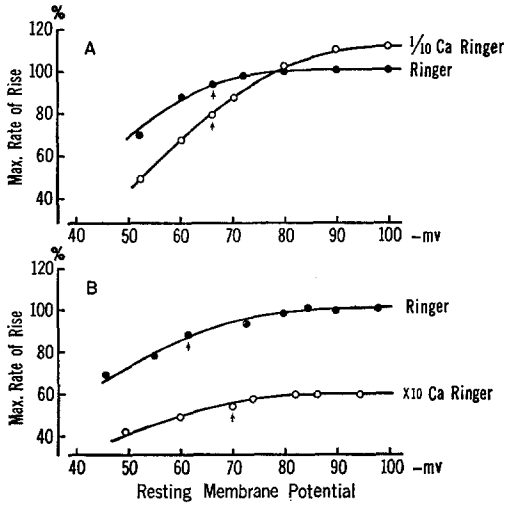

FIGURE 5. Effects of varying the Ca concentration in the external solution on the maximum rate of rise of action potentials of ganglion cells, produced at different membrane potential levels; the membrane potential was altered by applying conditioning anodal or cathodal currents (resting membrane potentials are shown by arrows). Abscissa, membrane potential at which action potentials were produced. Ordinate, relative values of maximum rate of rise of action potentials, scaled in percentage. The maximum rate of rise of action potentials, produced at a membrane potential at which Na inactivation was fully depressed in Ringer solution, was taken to be 100%. Duration of conditioning pulses was approximately 300 msec. A, obtained from a cell during perfusion with Ringer solution and low Ca Ringer solution. B, obtained from another cell in Ringer and in high Ca Ringer solution.

conductance. Qualitatively comparable results were obtained with the present experiment in which the maximum rate of rise as well as the peak level of the action potentials in a high or low Ca Ringer solution (see Methods) was studied at various resting potential levels.

The maximum rate of rise and the peak level of the action potentials produced at a constant resting membrane potential were changed when the external perfusing solution was altered from the modified Ringer to either a high (18 mM) or low Ca (0.18 mM) Ringer solution (see Methods). A decrease in the external Ca concentration from 1.8 to 0.18 mM decreased the maximum rate of rise at a given resting membrane potential level lower than approxi-

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mately −70 mv (Fig. 5 A). The maximum rate of rise of the action potentials produced in the low Ca Ringer solution, however, was larger than that in the modified Ringer solution at a relatively high resting membrane potential level where the Na inactivation was completely depressed (Fig. 5 A). In this case, the peak level of the action potentials showed no appreciable or only occasionally a slight increase in the low Ca Ringer solution. When the external Ca concentration was increased from 1.8 to 18 mM, the maximum rate of rise (Fig. 5 B) as well as the peak level of the action potentials was markedly depressed at any given resting membrane potential level.

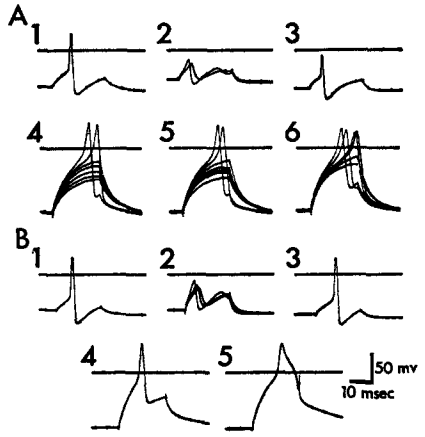

FIGURE 6. Effects of TTX (A) and procaine (B) on the Na (records 1–3) and Ca spikes (records 4–6) recorded from four different ganglion cells. A, records 1 and 2 were taken 3 min prior to and after addition of TTX (5×10^{-7} g/cc) to Ringer solution, respectively, and record 3 was taken approximately 30 min after withdrawal of TTX. Records 4, 5, and 6 were taken 3 min prior to and after, and 7 min after addition of TTX (5×10^{-6} g/cc) to an isotonic $CaCl_2$ solution, respectively. B, records 1 and 2 were taken 2 min prior to and after addition of procaine (5×10^{-3} g/cc) to Ringer solution, respectively, and record 3 was taken approximately 10 min after withdrawal of procaine. Records 4 and 5 were taken 5 min prior to and after addition of procaine (5×10^{-3} g/cc) to an isotonic $CaCl_2$ solution.

5. *Pharmacological Properties of the Ca Spikes*

It has been shown that the Ca spikes of crustacean muscle fibers (Hagiwara and Nakajima, 1965, 1966; Ozeki and Grundfest, 1965) or molluscan nerve cells (Meves, 1967; Chamberlain and Kerkut, 1967) are insensitive to TTX. This difference in the sensitivity to TTX was observed between the Na spikes and the Ca spikes of the present preparation. When a ganglion was perfused with Ringer solution containing TTX in a concentration of 5×10^{-7} g/cc, the Na spikes were readily depressed, and eventually disappeared within 5 min, while the resting membrane was gradually depolarized (cf. Fig. 6 A,

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record 2); the Na spikes could not be initiated at this stage even when the resting membrane was hyperpolarized by a conditioning anodal current. In the case of the Ca spikes, the cells maintained excitability without showing any appreciable drop in the resting potential when the ganglion was perfused with an isotonic $CaCl_2$ solution containing TTX in a concentration up to 5×10^{-6} g/cc. (Fig. 6 A, records 4–6).

As shown with crustacean muscle fibers (Hagiwara and Nakajima, 1966),

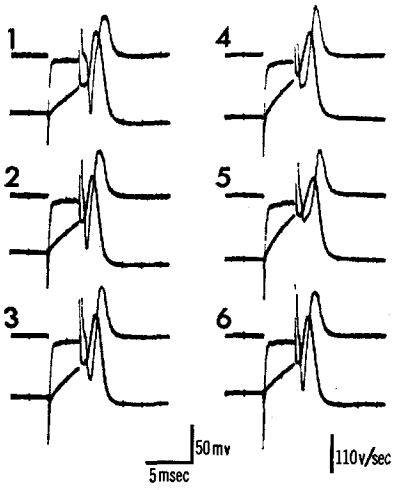

FIGURE 7. Effect of addition of Ca ions (30 mM) to a solution containing Na ions (57 mM) on the action potential of a ganglion cell. Record 1 was taken in Ringer solution, and record 2 approximately 3 min after the perfusate was changed to a solution containing 57 mM NaCl, 1.8 mM $CaCl_2$, and 112 mM sucrose. Records 3 and 4 were taken after approximately 1 and 3 min, respectively, in a solution containing 57 mM NaCl, 30 mM $CaCl_2$, and 30 mM sucrose. Record 5 was taken approximately 3 min after addition of TTX (5×10^{-6} g/cc) to this solution, and record 6 was taken again in the solution used for record 2. Note the decrease in the maximum rate of rise and the increase in the peak level of the action potential in record 3: The peak level measured from records 2 and 3 was +25 mv and +28 mv, respectively. The trace for the electrically differentiated potential changes represents the zero potential level. All solutions contained 2 mM K ions.

the Ca spikes of ganglion cells were resistant to procaine, although their falling phases were markedly prolonged (Fig. 6 B, records 4 and 5). When the same amount of procaine was added to Ringer solution, the cells rapidly lost excitability with a slight drop in the resting potential (cf. Fig. 6 B, record 2).

6. *Ca Spikes in Solutions Containing Na*

The present results indicate that Ca ions are acting as charge carriers during the production of the action potentials (Ca spikes) in a solution containing only Ca, K, and Cl ions. Ca ions were also capable of acting as charge carriers

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in a solution containing Ca and Na ions; the action potentials in this case consisted of Ca spikes as well as Na spikes. In Fig. 7 record 2 was taken in a solution containing 57 mM Na and 1.8 mM Ca ions. The perfusate was then switched to a solution containing 57 mM Na and 30 mM Ca ions, and records 3 and 4 were obtained. In the latter solution, the maximum rate of rise was markedly decreased, while the peak level of the action potential was not appreciably altered or slightly increased. When 16.2 mM Ca was added to the modified Ringer solution containing 87.7 mM Na, the maximum rate of rise as well as the peak level of the action potentials was seen to be markedly depressed (see section 4 under Results). The fact that the peak level showed no decrease while the maximum rate of rise of the action potentials was markedly depressed could be explained if the action potentials produced in the solution containing relatively high Ca and relatively low Na ions consisted of not only the Na spikes but also the Ca spikes. If TTX (5×10^{-6} g/cc), which blocks Na spikes without affecting Ca spikes (see section 5 under Results), was added to

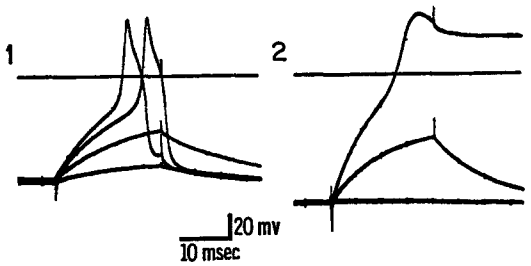

FIGURE 8. Sr spikes (1) and Ba potential (2) recorded from two different ganglion cells in isotonic $SrCl_2$ and $BaCl_2$ solutions, respectively.

this solution, the maximum rate of rise of the action potentials was further decreased without any appreciable change in the peak potential level (Fig. 7, record 5). This indicated that the component of the Na spike was blocked and only the component of the Ca spike remained in the presence of TTX.

7. *Action Potential in Sr Solution*

In an isotonic $SrCl_2$ solution, the present preparations were capable of producing action potentials (Sr spikes) (Fig. 8, record 1) which were similar to Ca spikes in configuration. In an isotonic $BaCl_2$ solution, they produced prolonged action potentials (Ba potentials), which often lasted for more than a few seconds (Fig. 8, record 2). The cells, however, were rendered inexcitable when the ganglion was perfused with an isotonic $MgCl_2$ solution for more than 10 min.

A. SR SPIKES. The values of the resting potential and the effective membrane resistance of cells perfused with the isotonic $SrCl_2$ solution were almost the same as those in the isotonic $CaCl_2$ solution; the former ranged from −80

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to −100 mv (among eight cells), and the mean value of the latter (among eight cells) was 167 ± 12 MΩ (SE of mean). The threshold membrane potential and the peak potential levels of the Sr spikes ranged from −10 to −25 mv and from +40 to +55 mv, respectively (among eight cells). The maximum rate of rise of the Sr spikes varied largely according to individual cells; its mean value (78 v/sec among eight cells) was 52 % of that of the Na spikes (20 cells). As observed with the Ca spikes, the Sr spikes could be elicited repetitively by a strong cathodal current of long duration, and seemed to be produced only from part of the cell body membrane. As also observed with the

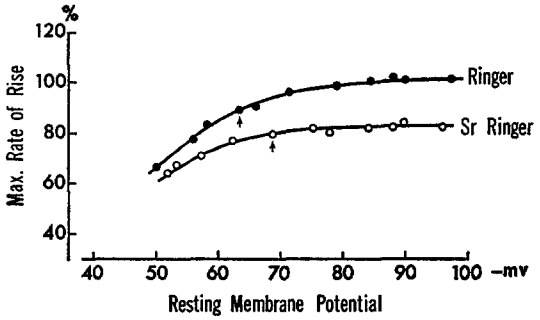

FIGURE 9. Effect of Sr ions on the maximum rate of rise of Na spikes of a ganglion cell. Ordinate, relative values of maximum rate of rise of Na spikes, scaled in percentage. The maximum rate of rise of the Na spikes, produced at a membrane potential at which Na inactivation was fully depressed in Ringer solution, was taken to be 100%. Abscissa, membrane potential at which Na spikes were produced. See text for the ionic composition of Ringer and Sr Ringer solutions. The resting membrane potentials (no conditioning anodal or cathodal currents) are shown by arrows. Duration of conditioning pulses was approximately 300 msec.

Ca spikes, the peak potential level of the Sr spikes was dependent on the external Sr concentration; a one-tenth decrease in the external Sr concentration showed roughly a 30 mv decrement of the peak level. The Sr inactivation occurred in the $SrCl_2$ solution when the membrane was depolarized to a potential level below approximately −70 to −80 mv by applying conditioning cathodal currents. The Sr spikes in the isotonic $SrCl_2$ solution were maintained without any appreciable change in the resting potential in the presence of TTX in a concentration of 5×10^{-6} g/cc. The Sr spikes were also resistant to procaine (5×10^{-3} g/cc), although their falling phase was prolonged.

B. EFFECTS OF SR IONS ON NA SPIKES Sr ions, like Ca ions, decreased Na inactivation at a fixed membrane potential level and decreased the maximum rate of rise of the Na spikes produced at a high resting membrane potential level at which Na inactivation was completely depressed (Fig. 9). In this experiment, the action potentials were first recorded from a cell in the modified

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Ringer solution (cf. Methods), and then in a solution which was prepared by replacing 18 mM $CaCl_2$ of the high Ca Ringer solution with equimolar amounts of $SrCl_2$. Similar to Ca ions, Sr ions were capable of acting as charge carriers in a solution containing both Na (57 mM) and Sr (30 mM) ions.

C. EFFECTS OF CA IONS ON SR SPIKES Ca ions were able to act on the Sr spikes, as they did on the Na spikes, in that the Sr spikes could be depressed by addition of Ca ions to the external solution. As seen in Fig. 10 A, the maximum

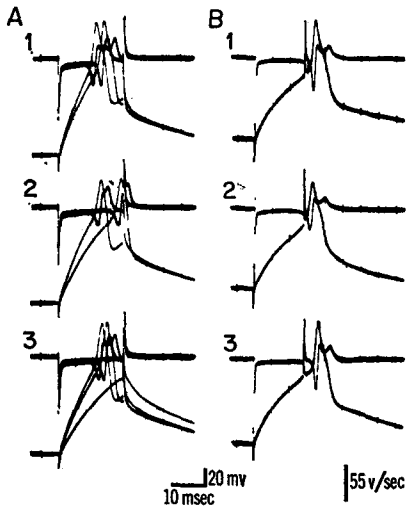

FIGURE 10. Interaction between Ca and Sr ions obtained from two different cells. A, records 1 and 2 were taken 3 min before and after 17 mM Ca ions were added to a solution containing 42 mM Sr ions, respectively, and record 3 was taken approximately 5 min after withdrawal of the Ca ions. All records were taken while the membrane potential was maintained at a level higher than −100 mv by applying anodal current, in order to completely depress Ca or Sr inactivation. B, records 1 and 2 were taken 3 min before and after withdrawal of 42 mM Ca ions from a solution containing 42 mM Sr and 42 mM Ca ions, respectively, and record 3 was taken approximately 5 min after the addition of 42 mM Ca ions. The trace for electrically differentiated potential changes represents the zero potential level.

rate of rise of the Sr spikes produced in a solution containing 42 mM $SrCl_2$ and 2 mM $KHCO_3$ (records 1 and 3) was decreased by addition of 17 mM Ca ions to the solution (record 2); the isotonicity of these solutions was maintained by sucrose. Addition of 17 mM Sr ions to a solution containing 42 mM $CaCl_2$ and 2 mM $KHCO_3$, however, did not bring about any appreciable changes in the maximum rate of rise or in the peak level of the Ca spikes. Withdrawal of Ca ions from the solution containing 42 mM Sr and 42 mM Ca brought about a marked reduction in peak height and rate of rise of the action potential (Fig. 10 B).

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DISCUSSION

The present experiment demonstrated that bullfrog sympathetic ganglion cells, like arthropod or molluscan cells (see Introduction for references), are capable of producing Ca spikes in a solution containing only $CaCl_2$ and KCL or $KHCO_3$. Furthermore, the present experiment suggested that Na as well as Ca ions are able to act as charge carriers during the action potential produced in a solution containing relatively high Ca and relatively low Na ions. The changes in the maximum rate of rise and peak level of the action potentials which were observed when the external Ca concentration was reduced or increased in Na-containing solutions could be explained by the fact that increasing external Ca concentration shifts along the voltage axis the curves that relate the activation or the inactivation of the Na conductance to the membrane potential (Weidmann, 1955; Frankenhaeuser and Hodgkin, 1957; Hille, 1968). These results indicate that Ca ions exert dual actions on the membrane of ganglion cells, namely, they are capable of regulating the changes in the membrane permeability to Na ions and also to act as charge carriers during the production of the action potentials of ganglion cells.

It has been assumed in our working hypothesis that the changes in the membrane permeability to Na, K, or other ions are caused by the changes in the structural or electrochemical nature of the membrane, which take place as a result of the dissociation of bound Ca in the membrane (Koketsu, 1965). According to this hypothesis, the increase in the membrane permeability to Na as well as to Ca or Sr ions during the action potentials is a function of the amount of the net loss of the bound Ca, which is caused by the Ca dissociation reaction triggered by stimulating cathodal currents (Frankenhaeuser and Hodgkin, 1957). If we assume that the dissociation of bound Ca is an ion exchange reaction, the net loss of the bound Ca would be proportional to the concentration of the bound Ca and inversely proportional to that of the free Ca in the external solution; the amount of the net loss would be increased when the concentration of bound Ca is high and that of the free Ca is low. When the external Ca concentration was reduced from 1.8 to 0.18 mM the maximum rate of rise of the action potentials was depressed at a given resting membrane potential level lower than approximately -70 mv. This can be explained by the shift of the inactivation curve along the voltage axis (Frankenhaeuser and Hodgkin, 1957), and may be due to a decrease in the external Ca concentration which decreases the concentration of bound Ca during the resting state of the membrane, consequently causing Na inactivation. It has been shown in the present experiment that Sr ions are able to depress Na inactivation, which suggests that bound Sr can substitute for bound Ca. An increase in the Ca or Sr concentration in the external solution was also ex-

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pected to depress the Ca or Sr inactivation. This, however, was not observed in solutions containing various amounts of Ca or Sr ions (8.4–84 mM). Presumably, the bound Ca (or bound Sr) was saturated when the concentration of Ca or Sr ions in the external solution was more than 8.4 mM. When Na inactivation was fully depressed by applying conditioning anodal currents, which would increase the concentration of bound Ca (Koketsu, 1965; and see also Frankenhaeuser and Hodgkin, 1957), the increase in the external Ca or Sr concentration depressed the maximum rate of rise and the peak level of the Na spikes. This can be explained by the shift of the Na conductance–membrane potential curve along the voltage axis (Frankenhaeuser and Hodgkin, 1957), and may be due to the possibility that an increase in the external Ca or Sr concentration prevented the net loss of bound Ca (or bound Sr) at a certain membrane potential level during the production of the action potentials. It was also shown in the present experiment that addition of Ca ions to the external solution depressed the increase in the membrane permeability to Sr ions during the Sr spikes, whereas addition of Sr ions did not depress the Ca permeability during Ca spikes. These results suggest that the binding force of Ca ions to the membrane was much stronger than that of Sr ions. It is likely that the increase in the membrane permeability to Ca or Sr ions during the Ca or Sr spikes, respectively, is depressed when the external Ca or Sr concentration is high. If this were the case, the membrane permeability to Ca or Sr ions during the Ca or Sr spikes, respectively, would be decreased according to the increase in the Ca or Sr concentrations.

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