The Effects of External Sodium and Potassium Concentration on the Membrane Potential of Atrioventricular Fibers of the Toad

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ABSTRACT   Intracellular records were made from fibers in the A-V conducting system of isolated toad hearts. The A-V region was perfused with Ringer's solution of various K and Na concentrations. Resting potential in 2.8 mM $[K]_o$ was about 60 mv. Over the range 0.28 to 28 mM, resting potential diminished with increasing $[K]_o$. Spontaneous action potentials appeared when $[K]_o$ was increased to 11.2 mM, and when resting potential had fallen to about 40 to 50 mv. Changes in $[Na]_o$ over the range 22 to 110 mM had a little effect on resting potential, but there was a linear relation between the peak value of the action potential and log $[Na]_o$. Wenckebach periodicity was observed when $[Na]_o$ was lowered.

The nature of atrioventricular (A-V) conduction has been under consideration for many years. Recently, it has become possible to record potentials from the cells in and near the A-V node with intracellular electrodes, and studies of this sort carried out on the rabbit have shown that there is a special region in which conduction velocity is extremely low (0.02 to 0.05 m/sec) and in which the action potentials rise slowly and are of low amplitude. Hoffman, Paes de Carvalho, De Mello, and Cranefield (1959) refer to this as the "A-V nodal region," while Paes de Carvalho and De Almedia (1960) have suggested the term "N layer."

Hoffman and coworkers have concluded that impulse conduction in the A-V nodal region is decremental, in the sense that the properties of the fibers change along their length in such a manner that the action potentials become progressively less effective as stimuli to the unexcited portions of the fibers ahead of them (Hoffman et al., 1959; Hoffman and Cranefield, 1960; Hoffman, 1961). One of the main arguments they present in support of their view is that the fibers in the A-V nodal region are resistant to the depolarizing action of high extracellular concentrations of potassium (Hoffman and Paes

de Carvalho, 1962). But, in fact, they report no observations on the effects of potassium on fibers in the A-V nodal region. Their observations with potassium were confined to fibers in the lower node (De Mello and Hoffman, 1960) where conduction was found to be nondecremental. Moreover, the potassium used to depolarize was added to the solution bathing the heart, a procedure that may not have assured a sufficiently rapid penetration of potassium to the lower node.

In the present study, experiments have been done on the A-V conducting fibers of the toad heart, a preparation in which rapid and efficient changes in the extracellular composition of the fluid around the A-V fibers can be made by perfusing the coronary vessels distributed to the A-V ring muscle. The results have yielded no evidence that the fibers of the A-V system are resistant to the depolarizing action of excess potassium. A short account of some of the findings was given at the 159th Tokyo Physiological Conference (Kanno, 1964).

METHODS

The experiments were performed on the isolated hearts of toads (*Bufo vulgaris formosus*) weighing from 100 to 300 g. The heart was perfused by the Langendorff technique through a cannula inserted into the aorta after the heart had been opened by a longitudinal cut from the atrium through the ventricle as described previously (Kanno, 1963 *a*). This cut did not interfere with perfusion of the dorsal (main) A-V pathway since the dorsal branch of the coronary vessels remained intact. Solutions of different ionic composition were led via separate tubes to a common junction near the input cannula (Fig. 1). The total dead space including the aorta was approximately 0.5 ml. The half-time for change of solution was different for each preparation, but it was estimated to be about 2 min from the observation of the rate at which the amplitude of the action potential changed in a solution in which the sodium concentration was 70% of normal. The marked differences in the amplitude and shapes of action potentials from different fibers in the A-V conducting system of the toad made it necessary to study the effects of any given ionic change on the same fiber, a procedure that demanded recording be maintained for prolonged periods. Since contractions of the atrial and ventricular muscles would have rendered this difficult, these contractions were curbed by immersing the preparation in Ca–free Ringer's solution while the A-V conducting pathway was independently perfused with Ringer's solution. The composition of the Ringer solution used for perfusing the conducting system (perfusion solution) was as follows (mM): NaCl, 110; KCl, 2.8; CaCl$_2$, 1.7; NaHCO$_3$, 2.4. The solution in which the rest of the heart was bathed (bathing solution) was the same except that CaCl$_2$ was omitted. When the Na concentration of a perfusion medium was altered, osmolarity was maintained with sucrose. No osmotic compensation was made for a small change in K concentration. The various solutions were equilibrated with pure oxygen, and the pH of these solutions ranged from 7.0 to 7.3 after oxygenation. The experiments were carried out at room temperature (15–18°C).

The degree of intermixing of the perfusion solution with the bathing solution was tested in control experiments by adding India ink or methylene blue to either one or the other of the two solutions. They were then examined microscopically. After perfusion, the heart was fixed with 10% formalin, dehydrated with ethyl alcohol, and immersed in methyl benzoate. Transparency of the preparation in methyl benzoate allowed visualization of the fine distribution of the coronary system, which contained India ink. When the bathing solution contained India ink (or methylene blue), there was no visible distribution of dye in the coronary system. After perfusion with either of the two substances, the dye could be observed throughout the vessels that are distributed exclusively in the A-V region (Fig. 2).
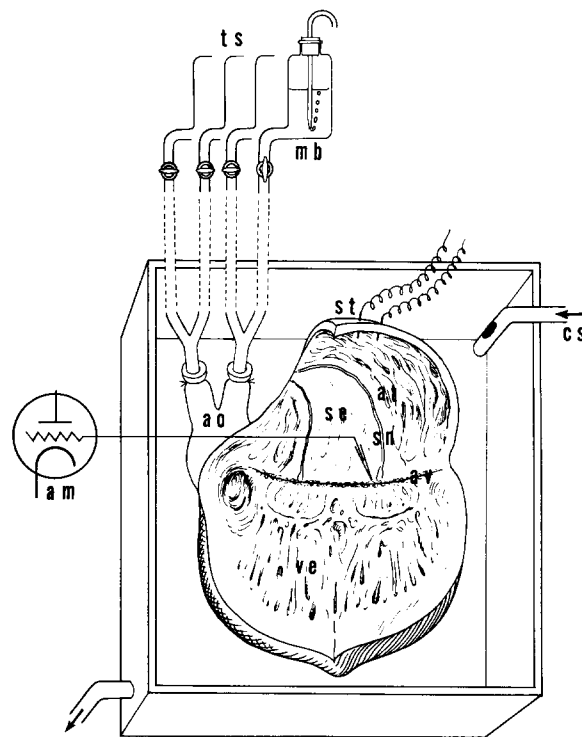

FIGURE 1. Schematic representation of the experimental arrangement. The A-V valves have been removed. *ao,* aorta. *am,* amplifier. *at,* atrium. *av,* atrioventricular ring muscle. *cs,* Ca-free Ringer's solution. *mb,* Marriott's bottle. *se,* atrial septum. *sn,* septal nerve. *st,* stimulating electrode. *ts,* test solution. *ve,* ventricle.

The heart was stimulated with rectangular pulses once every 5 sec through a pair of Ag-AgCl needle electrodes stuck into the atrium. Glass microelectrodes filled with 3 M KCl were used for intracellular recording. Their resistance ranged from 8 to 20 MΩ. The recording system consisted of a cathode follower input, dc amplifier, and cathode ray oscilloscope.

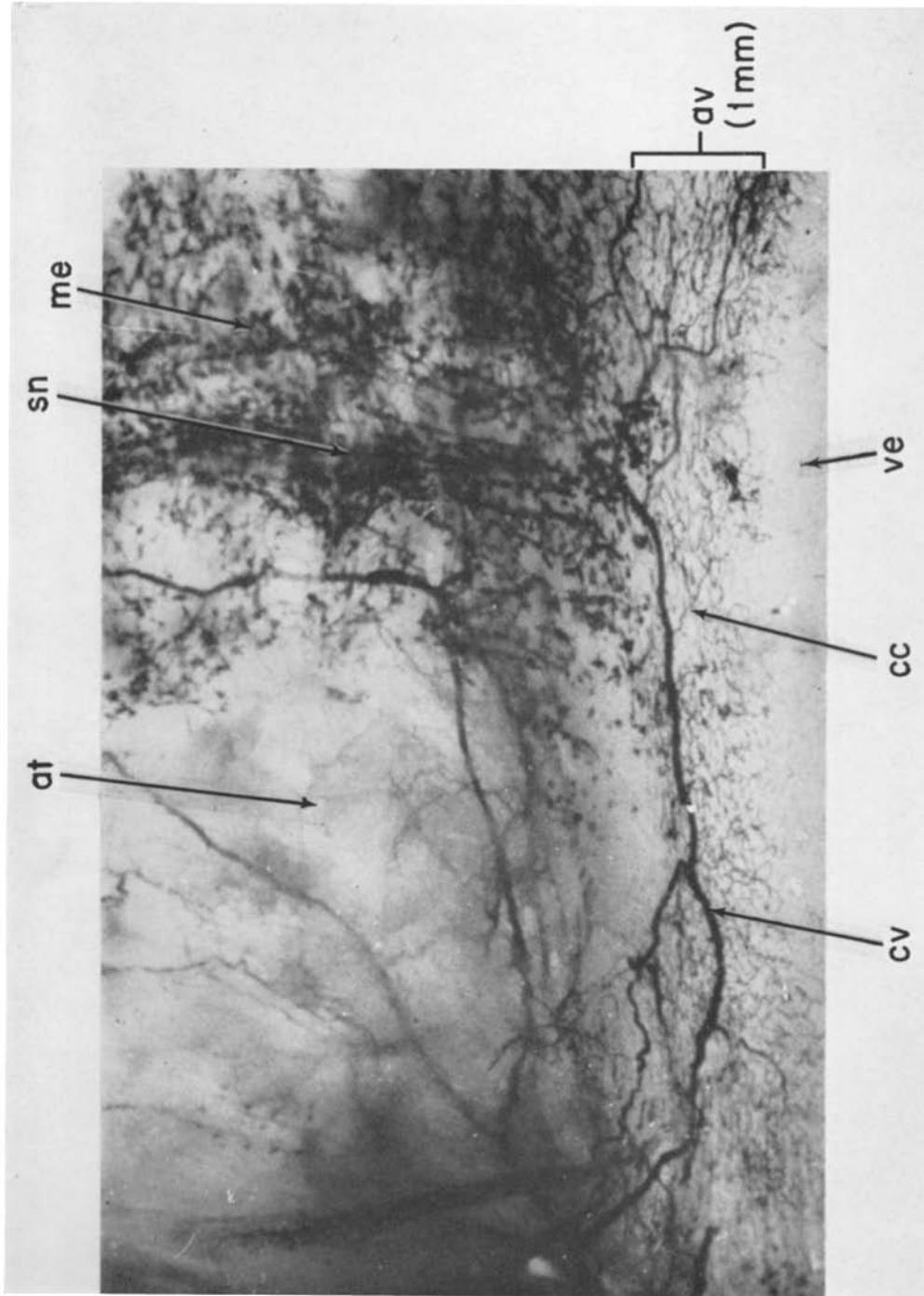Intracellular recordings were obtained from fibers lying in the A-V conducting

FIGURE 2. Photograph of a portion of toad heart after perfusion of the coronary vessels with India ink. India ink distributed in only the coronary circulation system which was confined to the A-V region. *at*, atrium. *av*, atrioventricular ring muscle. *cc*, coronary capillary network. *cv*, coronary vessels. *me*, melanophore. *sn*, septal nerve. *ve*, ventricle.

system adjacent to the atrium and close to the dorsal Bidder's ganglion. A micro-manipulator was used to carry microelectrodes under visual control. The fibers that were selected for study all showed characteristic changes in their action potentials upon stimulation of the septal nerve; such stimulation always lowered the amplitude of the action potential, decreased its rate of rise, and shortened its duration. These characteristic changes of the potentials are presumably due to the action of acetylcholine which is released from the cholinergic fibers included in the septal nerve (Kanno, 1963 *b*). These changes are among the important criteria used to identify the special A-V region of the lower vertebrates corresponding to the "A-V node" or N layer of mammals (Cranfield, 1965). The fact that fibers in this region have
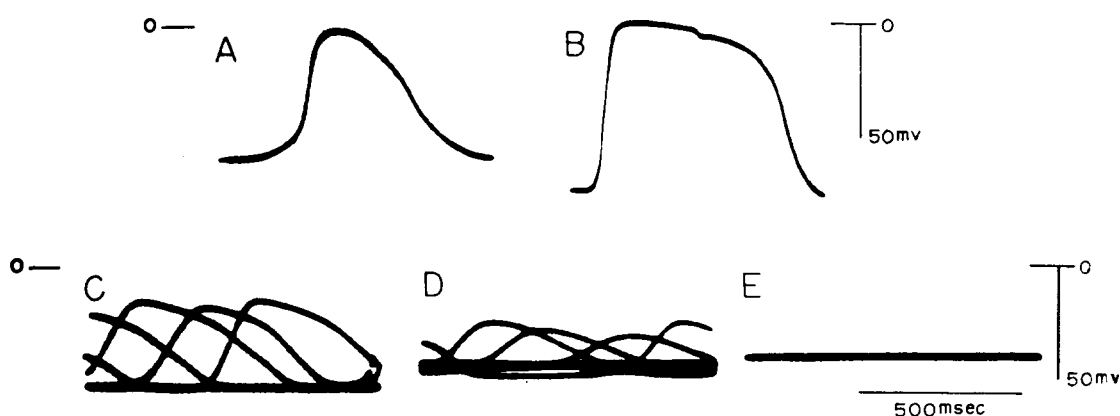

FIGURE 3. Effects of external K concentration on the action potential of an A-V conducting fiber in the toad heart. *A*, control in 2.8 mM $[K]_o$. *B*, in low $[K]_o$ (0.28 mM), resting potential is increased and so is the amplitude and duration of the action potential. *C*, in high $[K]_o$ (11.2 mM), resting potential is reduced and the action potential is of lowered amplitude and spontaneous excitation is present. *D*, in still higher $[K]_o$, (28 mM), the effects are further exaggerated and finally (*E*) the action potential disappears.

an extremely low conduction velocity (0.002 to 0.005 m/sec at room temperature) represents another similarity to the A-V node. The method for locating these fibers has been described in detail elsewhere (Kanno, 1963 *b*).

RESULTS

Effects of Potassium   The control response shown in Fig. 3 (record *A*) is typical of those obtained from the A-V fibers studied in the present experiments; the resting potential is low (about 60 mv), and the action potential rises slowly and is of relatively short duration. When coronary perfusion was switched from Ringer's solution with 2.8 mM K to a modified Ringer solution containing one-tenth of this concentration of K, the resting potential increased and so also did the rate of rise of the action potential, its amplitude, and dura tion (Fig. 3, record B). On the other hand, when the K concentration ($[K]_o$) was increased to 11.2 mM, the resting potential fell and the rate of rise of the

action potential was reduced, without, however, any marked change in the duration of its plateau (Fig. 3, record C). With this elevated concentration of K, the fiber discharged spontaneously as soon as its resting potential had been reduced to a critical level which ranged from 45 to 44 mv in the different preparations. When the K concentration was raised to 28 mM, the resting potential fell further and the changes in the action potential progressed (Fig.3, record D). Finally, when the resting potential had been reduced to about 40 mv, spontaneous action potentials disappeared (Fig. 3, record E). When
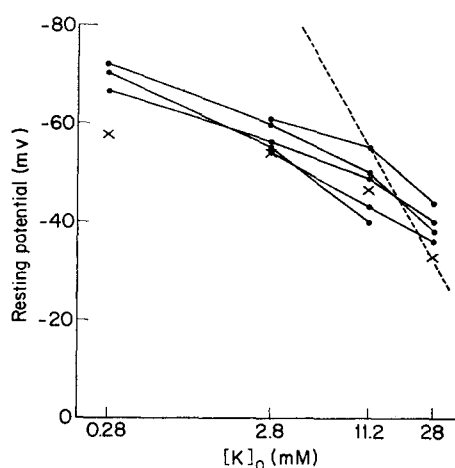

FIGURE 4. Membrane potential of A-V conducting fibers as a function of $[K]_o$. Some of the values were obtained from the experiment shown in Fig. 2. Others were derived from similar experiments on other hearts. The broken line indicates the K concentration potential drawn through an assumed $[K]_i$ concentration of 100 mM with a slope of 58 mv/decade. Points represented by cross (×) indicate theoretical values calculated from the equation presented by Hodgkin and Katz (1949). Temperature 18°C.

perfusion with the conventional solution containing 2.8 mM K was resumed, the whole sequence of change was reversed. Complete recovery occurred in about 10 to 15 min by which time the resting and action potentials were usually indistinguishable from the controls. The relation between $[K]_o$ and membrane potential calculated from the experiment of Fig. 3 has been plotted in Fig. 4 along with values obtained from other similar experiments. In the figure, a line with a slope of 58 mv per tenfold increase in the $[K]_o$ is drawn through the point representing 100 mM/liter intracellular water, and each of the points represented by a cross (×) indicates a theoretical value calculated from the modification of the Nernst equation presented by Hodgkin and Katz (1949).

Effects of Sodium   When the sodium concentration of the coronary perfu-

sion medium ([Na]$_o$) was reduced from the conventional value of 110 to 77 mM, the resting membrane potential was unaltered; but the action potential showed a decreased rate of rise and its amplitude was diminished (Fig.5, record *B*). When the [Na]$_o$ was further reduced to 44 mM, the first effect observed was the appearance of the electrical events associated with Wenckebach periodicity (Fig. 5, record *C*), i.e. the interval between atrial excitation and the electrical response of the A-V fiber progressively lengthened in successive
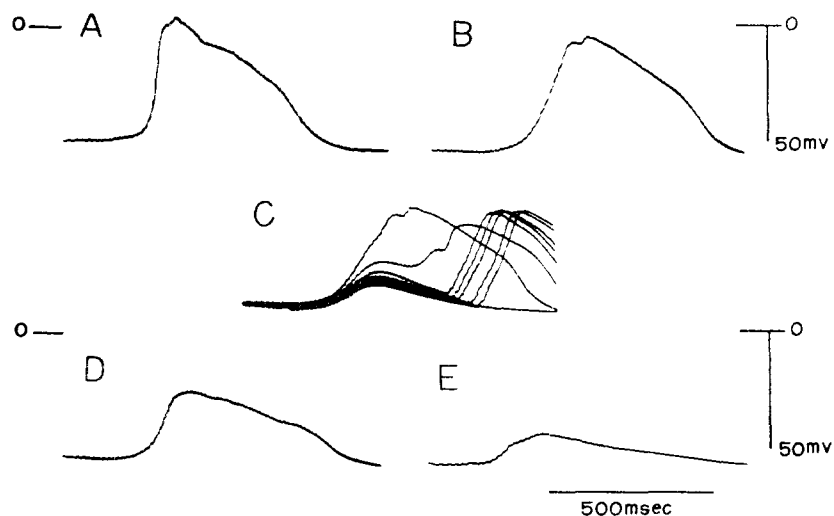

FIGURE 5. Effects of lowered external Na concentration on the action potential of an A-V conducting fiber in the toad heart, sodium being replaced with sucrose. *A*, control in 110 mM [Na]$_o$. *B*, in 77 mM [Na]$_o$. *C*, emergence of Wenckebach periodicity in the course of changing [Na]$_o$ from 77 mM to 44 mM. *D*, later in 44 mM [Na]$_o$ the action potential has become stable. *E*, in 22 mM [Na]$_o$.

cycles until it became so long that the A-V fiber failed to respond. Then, following this failure of response, the whole cycle was repeated (see Paes de Carvalho and De Almeida, 1960) and commonly during this period of instability, the upstroke of the A-V action potential showed several steps as in *C* of Fig. 5. The Wenckebach periodicity was, however, a transient phenomenon which always disappeared some 15 min after starting perfusion with the solution containing 44 mM Na. After this, the action potential became stable again with a greatly lowered rate of rise and a much reduced amplitude (Fig. 5, record *D*). Under these conditions conduction was blocked as shown by the disappearance of the extracellularly recorded ventricular potential changes. The much reduced A-V action potentials, of which record *D* (Fig. 5) is typical, can thus be regarded as local responses. When the [Na]$_o$ was reduced to 22 mM, still smaller local responses of the type shown in record *E* (Fig. 5) were ob-

tained. The sequence of changes shown in Fig. 5 was irreversible over 2 hr. The resting membrane potential was well maintained in all Na-poor media.

The effect of $[Na]_o$ on the peak value of the action potentials of A-V fibers is plotted in Fig. 6 along with the line representing the theoretical depolarization assuming as intracellular sodium concentration of 110 mM, a value equal to the sodium concentration of the Ringer solution, and with a slope of 58 mv per tenfold increase in Na concentration. The experimental results fit this calculated relation fairly well.
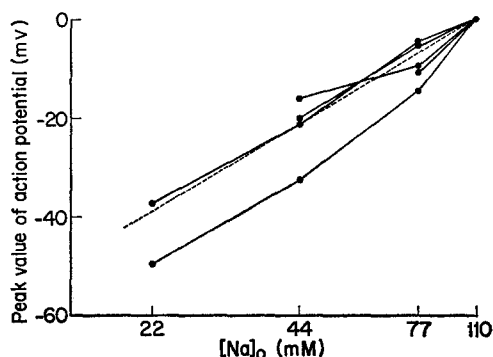

FIGURE 6. Peak value of action potential of AV conducting fibers as a function of $[Na]_o$. The values were obtained from the experiment shown in Fig. 4, and from other similar experiments. The broken line indicates the Na concentration potential drawn through an assumed $[Na]_i$ of 110 mM with a slope of 58 mv/decade. Temperature 18°C.

DISCUSSION

The present results obtained with potassium differ from those obtained by De Mello and Hoffman (1960) who found that a rise in $[K]_o$ from 2.7 to 21.6 mM had very little effect on the resting and action potentials from their preparations, and that action potentials still developed and appeared to propagate when the K concentration in the bathing medium was as high as 40.5 mM. By contrast, in the present experiments, a rise in $[K]_o$ to 11.2 mM was sufficient to produce a discernible lowering of action potentials in the A-V fibers and to initiate spontaneous action potentials whose rise time was much reduced and whose amplitude was diminished, and in 28 mM potassium, the resting membrane potential was still further reduced and action potentials disappeared. While it is conceivable that the discrepancies in results are attributable to species variations, or to differences between A-V and lower nodal fibers, it seems more likely that the differences are due to the fact that the test medium used by De Mallo and Hoffman was not in equilibrium with the extracellular environment of the fibers they studied. As noted earlier, these investigators did not perfuse the heart but simply bathed it with test solutions. Under such

conditions, the rate of penetration of K to the lower node, which has a dense investment of connective tissue, may have been slow. This possibility was rejected by De Mallo and Hoffman on the basis of several observations. However, their records showed that the effect of $[K]_o$ on the lower A-V node was much slower in onset than the effect on the atrium: the effect of 16.2 mM K on the atrium was definite after 20 min, whereas, there was little effect of 21.6 mM K on the lower node after 30 min, but a noticeable effect after 4 hr.

The effects of $[K]_o$ on resting and action potentials of A-V fibers of the toad in the present experiments are similar to the reported effects of $[K]_o$ on rabbit atrial, frog ventricular, and sheep Purkinje fibers (Hoffman, 1959; Brady and Woodbury, 1960; Weidmann, 1957). However, in the physiological and lower ranges of $[K]_o$, the estimated membrane potentials were far below the corresponding K equilibrium potentials calculated from the Nernst equation, when intracellular K concentration ($[K]_i$) was assumed to be 100 mM, which is the estimated value for fibers in the sinus venosus (Danielson, 1964). This discrepancy between the value calculated from the Nernst equation and the estimated value has been observed in other cardiac muscles; *i.e.*, sino-atrial node (De Mello and Hoffman, 1960), rabbit atrium (Hoffman, 1959), frog ventricle (Brady and Woodbury, 1960), and sheep Purkinje (Weidmann, 1959). Because the A-V fiber is thought to be in an unsteady state, the permeabilities to Cl and Na may also help to determine the resting potential, and we may use the modification of the Nernst equation presented by Hodgkin and Katz (1949) who propose that

$$ E = \frac{RT}{F} \log_e \left\{ \frac{P_k[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}{P_k[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i} \right\} $$

where E is the potential difference between the external solution and the inside of the fiber, P_K, P_{Na}, and P_{Cl} are the permeability constants for the individual ions, R, T, and F are the gas constant, absolute temperature, and Faraday constant, respectively. $[Na]_i$, $[Cl]_i$, and $[Cl]_o$ are the intracellular Na, Cl, and extracellular Cl ion concentrations, respectively. None of the intracellular ion concentrations and their permeability constants in the toad A-V conducting system is known at present. Therefore, $[K]_i$, $[Na]_i$, and $[Cl]_i$ are assumed provisionally to be 100 mM, 0 mM, and 24 mM, respectively (based on the corresponding values for fibers in the toad sinus venosus presented by Danielson, (1964). The relative values for the permeability coefficients are assumed to approximate those for *Loligo*: P_K: P_{Na}: P_{Cl} = 1:0.04:0.45 in the lower range of $[K]_o$ and P_K: P_{Na}: P_{Cl} = 1:0.025: 0.3 in the higher range of $[K]_o$ (Hodgkin and Katz, 1949). Fig. 4 shows that each of the theoretical resting potentials obtained on these bases coincides with each of the values obtained experimentally in the present study. In the same manner, the resting potential of A-V fibers in low $[Na]_o$ may be calculated, e.g. E = 51.6 mv

when $[Na]_o$ = 22 mM. Hence, the theoretical resting potential would be changed very slightly when $[Na]_o$ is lowered. Such was found to be the case in this study, and therefore, Na and Cl may participate in the maintenance of the resting potential in the toad A–V fibers.

The responses of the toad A-V fibers to a lowering of $[Na]_o$, which involved a prolongation of the rise time of the action potential and its amplitude without any change in resting potential, are similar to those found for other cardiac muscle fibers (Draper and Weidmann, 1951; Brady and Woodbury, 1960). The reduction in amplitude of the action potential with lowered $[Na]_o$, was in reasonable agreement with the prediction of the Nernst equation $[Na]_i$ was taken to be 110 mM. Such a high intracellular Na concentration would be in accord with some evidence that other specialized fibers in the heart have a high Na content. Thus, according to Davies et al. (1952) the A-V node of ox heart contains 155 mM Na/kg wet weight; and Mazel and Holland (1958) have reported that the sinus venosus of the frog and the turtle contains 124 and 129 mM Na/kg wet weight, respectively. However, Danielson (1964) has more recently suggested that the differences in the Na and K content of different regions of the toad heart are due to differences in extracellular volumes, and concluded that the $[Na]_i$ and $[K]_i$ are the same in different regions of the heart. If this is true, then an alternative explanation of the results shown in Fig. 5 might be that the rate of activation and inactivation of P_{Na} might be so exceptionally low in the A-V fiber that peak value of the action potential could not reach the sodium equilibrium potential. Such a hypothesis might also help to explain the following characteristics of the A-V fiber: a slowly rising action potential with little or no overshoot and no spike, and refractoriness outlasting the phase of repolarization.

The production of Wenckebach periodicity on lowering $[Na]_o$ does not seem to have been reported before. It may be a useful clue to the mechanism of this curious phenomenon not uncommonly encountered in anoxic hearts. Potentials in the Wenckebach periodicity consist of two components; the local response and the action potential. There are two types of action potentials; one shows a slow and notched rising phase and the other a relatively fast and smooth rising phase. In another experiment, when the former type of action potential was observed in A-V fibers of the toad in orthodromic conduction, it was commonly changed to the latter by antidromic activation from stimulated ventricular muscles (Kanno, unpublished observation). Hence, the former type may be orthodromically conducted and latter type antidromically conducted. When local response fails to excite A-V fibers, the A-V conducting system recovers from the refractory period and can respond to antidromically conducted impulses which orginate from preceding ventricular excitation ("reentry" phenomenon). The various delays between local response and the action potential may be related to instability of P_{Na}.

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