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혈관근육세포내에서의 Na^+과 K^+이온의 이동 현상에 대한 동역학적 해석

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Kinetic Analysis of Sodium and Potassium Ion Transfer in the Arterial Smooth Muscle Cells

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요 약

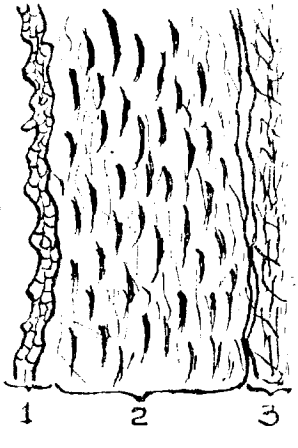
혈관근육의 수축과정에 관련된 기구를 알아 보고자 스테인레스강철대에 꿰인 개의 혈관도막을 사용하여 혈관벽내에서의 Na^+과 K^+ 이온들의 이동등특성을 연구하였다. 세포사이공간내에서의 확산과 능동적 및 피동적 기구에 의한 세포막투과를 함께 고려한 수학적 모형에 의거하여 추적자세척실험 데이터를 분석하였으며 그로부터 세포성질에 대한 수치를 얻었다. 세포사이공간내에서의 Na^+ 이온의 확산계수는 37°C에서 8.33×10^{-6} cm²/sec, 21°C에서 6.88×10^{-6}cm²/sec 이였고 37°C에서 K^+ 이온의 값은 1.23×10^{-5} cm²/sec이였다. Na^+이온의 세포막투과성은 37°C와 21.2°C에서 각각 0.60×10^{-8} cm/sec, 0.52×10^{-8} cm/sec이였고 37°C에서 K^+이온에 대한 값은 3.3×10^{-8} cm/sec였으며 Na^+이온의 능동적 세포막투과속도는 37°C와 21.2°C에서 각각 2.09×10^{-9} mEq/cm²sec, 1.52×10^{-9}mEq/cm²sec이였다. K^+이에온 대해서는 37°C에서 0.82×10^{-9} mEq/cm²sec의 값을 얻었다. 또한 37°C에서 Na^+이온과 K^+이온의 세포내 농도는 각각 18.7, 126 mEq/l이였고 21.2°C에서 Na^+이온의 값은 27.5 mEq/l였다. 이러한 결과는 $Na^+ - K^+$펌프는 연결비가 2 : 1로서 기전적(起電的)임을 시사하고 있으며 나아가 기전적 $Na^+ - K^+$펌프가 세포막전압을 유지하고 혈액흐름에 대한 혈관저항을 조절함에 있어 주요한 역할을 한다는 가정과 일치한다.

ABSTRACT

In an attempt to help elucidate the mechanisms involved in the contractile processes of vascular smooth muscle, the transport kinetics of Na^+ and K^+ in arterial walls (branches of canine femoral artery) were studied with isolated sections of vessels mounted on stainless steel rods. The experimental tracer washout data were analyzed based on a mathematical model, which takes into account diffusion in the extracellular space and transport across the cell membranes by both passive and active mechanisms, yielding values for the membrane properties. Values were obtained for the diffusion coefficient of the ions in the extracellular space ($8.33 \times 10^{-6}cm^2/sec$ at 37°C and 6.88×10^{-6} at 21.2°C for Na^+ and 1.23×10^{-5} at 37°C for K^+), the membrane permeabilities to the ions (0.60×10^{-8} cm/sec at 37°Cand 0.52×10^{-8} at 21.2°C for Na^+ and 3.3×10^{-8} at 37°C and K^+), the rate rate of active transport of ions through the cell membranes (2.09×10^{-9} mEq/cm² sec at 37°C and 1.52×10^{-9} at 21.2°C for Na^+ and 0.82×10^{-9} at 37°C for K^+), and the intracellular ion concentrations (18.7mEq/l at 37°C and 27.5 at 21.2°C for Na^+ and 126 at 37°C for K^+). The results indicate that the $Na^+ - K^+$ pump is electrogenic with a coupling ratio in the order of 2 to 1. This is consistent with the hypothesis that an electrogenic $Na^+ - K^+$ pump plays a role in maintaining the cell potential and in regulation of vascular resistance to blood flow.

1. Introduction

Mass transfer in the walls of blood vessels plays an important role in the process by which the body controls blood flow to individual organs. Smooth muscle cells located in walls of arterioles (see *Fig. 1*) contract or relax, changing the bore of these vessels and in turn regulating the resistance to blood flow in a vascular bed. In general, it is well established that changes in vascular caliber are normally associated with prior changes in the transmembrane potential of vascular smooth muscle (VSM) cells (i.e., depolarization is associated with a decreased caliber and hyperpolarization with an increased caliber) though the detailed mechanisms involed in the contractile processes are not yet known. The transmembrane potential of VSM cells is in turn maintained by an active transport of ions which is balanced in the steady state by diffusive trans-



1. Inner Coat
2. Middle Coat
3. Outer Coat

Fig. 1. Transverse section of wall of blood vessel

port of the ions under the influence of electrical and chemical driving forces. Any change in the environment of cells (such as changes in blood composition) can lead to changes in the active and passive fluxes of ions across the cell membrane, resulting in altered transmembrane potentials of the cells and subsequent contraction or dilation of the vessel.

In an effort to characterize the basic properties of ion transport mechanisms, many kinetic mass transfer studies have been done on isolated vascular smooth muscles as well as intact tissues. [1,3,5,7,12,13,15] Most of the experimental data have been interpreted based on a multicompartment model of the tissue which ignores diffusion in the extracellular fluid under the assumption that the only resistance to mass transfer between compartments is localized at the interface between them. Furthermore, no attempt was made to model the active transport process, though the overall fluxes have been evaluated.

Diffusion effects become important, especially when a whole muscle tissue is used for the kinetic study of ion exchange. It has been noted by serveral investigators that the rate of transfer depends upon the dimension of the sample tissue,[9,14] indicating that diffusion plays at least some part in the process.

In this study, the kinetics of transport of sodium and potassium ions in isolated arteries were examined both theoretically and experimentally, with the use of a radioactive tracer washout technique. Experimental washout data on Na^{22} and K^{42} effluxes from vascular tissue were analyzed based on a mathematical model which takes into account diffusion in the extracellular space and transport across cell membranes by both passive and active mechanisms. The specific aim of this work was to determine the membrane permeabilities of vascular smooth muscle cells to sodium and potassium ions and the active pumping rates of the ions. Cell potentials and the extra and intracellular volume fractions of the sample tissues were also measured under the same conditions as the washout studies to complement these data. The results are discussed based on the electrogenic $Na^+ - K^+$ pump hypothesis.

Ⅱ. Methods

(1) Tracer Washout Studies

Sections of a small branch of the femoral artery were removed from mongrel dogs weighing 20 to 30 kg, which were anesthetized by intravenous injection of sodium pentabarbiol (33mg/kg). The artery was mounted on a section of stainless steel rod slightly larger than the inside diameter of the vessel. In this manner the artery could be held rigid and under tension throughout the experiment to simulate as closely as possible physiologic conditions. The mounted tissue was cleaned and loose adventitia trimmed off with a samll pair of scissors in physiologic Ringer's solution. The length and outside diameter of the tissue at several locations were measured using a microcomparator and averaged, the inside diameter being determined by the size of the rod. The tissue was then incubated in a tagged physiologic Ringer's solution long enough to reach steady state concentration of tracer ions.

For the sodium washout study radioactive ^{22}Na as NaCl, was added to the incubating solution at a concentration of about $1.5\mu c/ml$ (10^{-3}mM/l) (not enough to alter the chemical concentration but sufficient to give

reasonable count rates). The experimental incubation was carried out in 10 m*l* of normal physiologic Ringer's solution bubbled with 95% O_2 and 5% CO_2 and mainained at 37°C or room temperature, depending on the experiment. Incubation periods of 90 minutes were used to insure equilibrium with ^{22}Na.[5]

The composition in mEq/1 of normal Ringer's solution was as follows:

$NaCl = 123.0$ Calcium Gluconate $= 4.8$
$NaHCO_3 = 23.0$ $MgCl_2 = 2.0$
$KCl = 4.2$ Glucose $= 1.0 g/l$

For the potassium washout study the mounted tissue was placed in K^+— free Ringers' solution saturated with the gas mixture of 95% O_2 and 5% CO_2 and maintained at 37°C for 90 minutes to deplete intracellular K^+ content. The tissue was then incubated in 10 m*l* of normal Ringer's solution made of ^{42}KCl solution (activity of about 30 μc). The incubating solution was bubbled with the 95-5% gas mixture and maintained at 37°C. Incubation periods of 3 hours were used to insure equilibration of the tissue with the ^{42}K. The potassium ion depletion during preincubation insured complete equilibration with the ^{42}K after incubation.

After being loaded with ^{22}Na or ^{42}K the tissue mounted on the rod was removed from the incubating solution and slightly blotted on tissue paper to clean the residual external fluid on the surface of the tissue. The rod with mounted tissue was then inserted into the washing chamber and the chamber was placed in the well of a 3″ NaI crystal detector.

The radioactive tracer ^{22}Na or ^{42}K in the tissue was then washed continuously with untagged Ringer's solution (saturated with the 95-5% gas mixture) in an apparatus designed to maintain constant temperature and constant flow throughout the entire washing period. Ringer's solution was pumped from a 15 liter constant temperature reservoir through the chamber, washing the outer surface of the artery with untagged solution. Constant flow was maintained with a pump. The bypass arrangement (see *Fig. 2*) allowed setting the apparatus at working condition before starting the washing of the tissue.

The internal volume of the chamber between the location of the tissue and the flow outlet was 0.4 m*l* and the flow rate was 3.0 m*l*/sec. Thus the residence time of the radioactive tracers was in the order of 0.13 sec after diffusing from the tissue. It was assumed that any mass transfer resistance at the outside surface of the tissue could be neglected at these flow conditions.

During the washout period the total radioacivity remaning in the tissue was monitored by counting for 30 sec time intervals. By using two scalers for the measurement, continuous recordings were possible without the interruption for printing. After the wash period, the washing chamber without the tissue was counted as a backround and this was subtracted from the original values.

(2) Extracellular and Intracellular Volume Measurement

A section of canine femoral artery was mounted on a stainless steel rod as previously described. The mounted artery was then weighed and the "wet weight" of the tissue obtained from the difference of this weight and the weight of the rod. The tissue was then incubated at 37°C in an oxygenated Ringer's solution containing ^{14}C-inulin. After a 30 minute incubation, when

the inulin had equilibrated between the extracellular space and the incubation bath, the tissue was removed from the bath, and the external moisture was blotted on a Kimwipe wiper. The artery, without the rod, was then placed in a known volume of water to wash out the labelled inulin. The concentration of inulin washed out of the artery was measured on a scintillation counter to determine the extracellular space.

The tissue was dried in an oven at 105°F for 24 hours to evaporate the water. The "dry weight" was then subtracted from the "wet weight" to give total water volume. The intracellular volume was found from the difference between total water and extracellular volume.

(3) Membrane Potential Measurement

Sections of femoral artery (about 25 mm long), obtained in the same manner as those for the washout study, were incubated in normal Ringer's solution saturated with a gas mixture of 95 % O_2 and 5 % CO_2 and maintained at 37°C for 1.5 hours. The composition of normal Ringer's was the same as that used for the washout study. During this period the tissue is believed to be equilibrated with the new ionic environment. After the incubation, the connective tissue was carefully removed using a small pair of scissors still in Ringer's solution. The tissue was then cut open along the longitudinal axis and held in position withthe inside upward in a recording chamber with an agar pad by four small pins. The chamber was irrigated with normal Ringer's solution and the temperature was kept consant at 37°C. The intracellular recordings of membrane potential were made with microelectrodes filled with 3M-KCl. Microelectrodes of 40-70 MΩ resistance were vertically inserted into the smooth muscle cells in the vessel walls using a hydraulic microelectrode driving system, which allowed a step movement at a variable rate with three different step sizes, 1μ, 5, and 10. The potentials were measured with reference to an indifferent electrode in contact with the

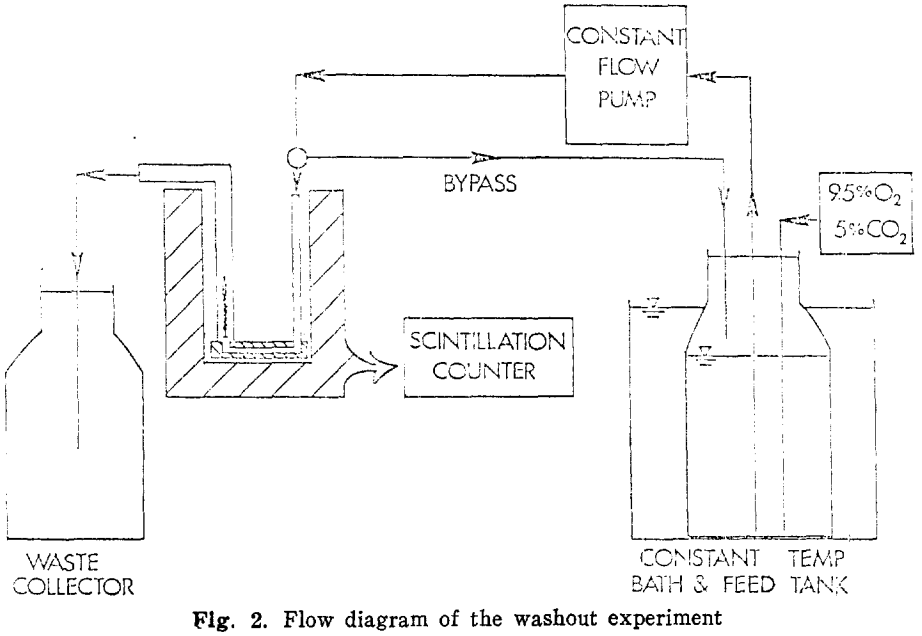


Fig. 2. Flow diagram of the washout experiment

bathing solution. The bathing solution was grounded. The negative potential was monitored on an oscilloscope and recorded on a moving chart.

Ⅲ. Theoretical Analysis

(1) Washout Process

A short section of artery, mounted on a stainless steel rod, is assumend to be made up of a collection of smooth muscle cells which exchange ions with the extracellular fluid. The ions can in turn diffuse in the extracellular space. During the washout period, tracer ions diffuse from the extracellular space to the washing solution and are carried away. Since the thickness of the tissue used for experiments was very small compared to the length, the diffusion process is assumed to occur only in the radial direction without an end effect.

Conservation equations in cylindrical coordinates can be written for any tracer ion species in both the intracellular and extracellular spaces, respectively.

$$\frac{\partial C_e^*}{\partial t} = D \cdot \left[\frac{\partial^2 C_e^*}{\partial r^2} + \frac{1}{r}\frac{\partial C_e^*}{\partial r} \right] - \frac{\varepsilon_i}{\varepsilon_e} \cdot a \cdot (K_1 C_e^* - K_2 C_i^*) \quad (1)$$

$$\frac{\partial C_i^*}{\partial t} = a \cdot (K_1 C_e^* - K_2 C_i^*) \quad (2)$$

where C_e^* and C_i^* refer to the extracellular and intracellular concentrations of tracer ion at radial position r; D is the effective diffusion coefficient in the extracellular space; ε_e and ε_i are the respective volume fractions of the tissue; t is time; K_1 and K_2 are mass transfer rate constants for influx and efflux of the ions; and a is the membrane area per unit volume of intracellular fluid.

When the tissue gets equilbrated with the tracer ions the specific activities in the extra- and intracellular fluids become equal to each other:

$$\frac{C_e^*(o)}{C_e} = \frac{C_i^*(o)}{C_i} \quad (3)$$

where $C_e^*(O)$ and $C_i^*(O)$ are the initial concentrations in the extra-and intracellular spaces, respectively, before the washing takes place. Rarranging Equation (3) gives

$$\frac{C_i^*(o)}{C_e^*(o)} = \frac{C_i}{C_e} \quad (4)$$

Since the tissue is in a steady state with respect to overall ion concentation, the concentration ratio, C_i/C_e, remains constant. This ratio is defined to be ϕ:

$$\frac{C_i^*(o)}{C_e^*(o)} = \phi \quad (5)$$

Then, the initial differential count rate, N*(O), obtained from the experimental result, becomes

$$N^*(o) = BV_t[\varepsilon_i C_i^*(o) + \varepsilon_e C_e^*(o)] \quad (6)$$

where B is a proportionality constant which relates the total Na^{22} ions to their count rate. Henceforth, this proportionality constant is incorporated into the concentration terms That is, the concentratinos, C_e and C_i^*, are treated as actual count rates equivalent to the concentrations.

Since the tissue mounted on a stainless steel rod is washed on the outer surface there is no flux across the inner surface. Also with a flow rate of 3.0 ml/sec through the space of about 0.4 ml any mass transfer resistance at the outer surfac of the tissue can be neglected.

Thus the initial and boundary conditions become:

$$C_e^* = C_e^*(0) \quad (7)$$
$$C_i^* = C_i^*(0) \quad t = 0 \quad (8)$$
$$\frac{\partial C_e^*}{\partial r} = 0 \quad r = R_a \quad (9)$$
$$C_e^* = 0 \quad r = R_b \quad (10)$$

The values of $C_e^*(0)$ and $C_i^*(0)$ are obtained

by solving Equations (5) and (6) simultaneously:

$$C_e^*(0) = \frac{N^*(0)}{V_t(\varepsilon_i \cdot \phi + \varepsilon_e)} \quad (11)$$

$$C_i^*(0) = \phi \cdot C_e^*(0) \quad (12)$$

Equations (1) and (2) were solved using numerical methods to give C_i^* and C_e^* in the tissue as functions of radial position and time. Since the instantaneous count rate (N^*) from the tissue is proportional to the amount of tracer ions in the tissue, the concentrations, C_e^* and C_i^*, are treated as actual count rates equivalent to the concentrations. Then N^* at any time is given by

$$N^* = \int_{Ra}^{Rb} 2\pi r L \cdot (C_i^* \varepsilon_i + C_e^* \varepsilon_e) \cdot dr \quad (13)$$

Where Rb and Ra are the outside and inside radii of the specimen. Since the experimental data from the washout are integral counts, $\bar{N}^*$, over specific time intervals, N^* must be integrated over the counting intervals in order to simulate the experimental values of $\bar{N}^*$. Again this integration was carried out numerically using the computer.

Thus for a given choice of parameters (D, K_1, K_2, ε_i, ε_e, E_m, $\underline{a}$) in the model, washout curves were simulated and compared to the experimental data. The parameters were then adjusted until a fairly good fit of the data was obtained. Then using these values as the initial guess, the parameters were finally optimized with a computerized optimization routine.

In order to minimize the number of parameters to be adjusted, $\underline{a}$ was taken to be 10^4 cm^2/cm^3 based on a smooth muscle cell of cylindrical shape with radius of 10^{-4}cm.[12] For the E_m, ε_e, and ε_i, values measured in this work were used ($E_m = 50$mV, $\varepsilon_e = 0.413$ and $\varepsilon_i = 0.347$). The diffusion coefficient was adjusted only through the preliminary optimizing process for each set of runs. The averaged value of each set was then used for the rest of the optimization. The adjustable remaining parameters in the model were the mass transfer rate constants, K_1 and K_2.

(2) Ion Fluxes Across Membranes of Vasclar Smooth Muscle Cells

In vascular smooth muscles there are both active and passive fluxes of an ion species across the cell membrane, which are balanced with each other in the steady state. The passive flux of an ion is best described by the constant field equation of Goldman.[2,6] For the tracer ions the equation becomes

$$j^*_{pass} = \frac{PE_mFC_e^* - PE_m\mathrm{Fe}^{-E_mF/RT}C_i^*}{RT(1 - e^{-E_mF/RT})} \quad (14)$$

where P is the membrane permeability to the ion, E_m is the membrane potential; R is the gas constant, T is the absolute temperature; F is the Faraday constant.

Defining the active rate of transport of an ion across the cell membrane per unit area per unit time to be S, the active flux of tracer ion across the cell membrane becomes

$$j^*_{act,K} = S_k \cdot \left(\frac{C_e^*}{C_e}\right) \quad (15)$$

$$j^*_{acr,Na} = S_{Na} \cdot \left(\frac{C_i^*}{C_i}\right) \quad (16)$$

where C_e and C_i are total extra-and intracellular concentractions of the ions (flux into the cell is taken as positive). It is assumed that sodium is only pumped out and potassium into the cell. Total flus is then

$$j_{tot,K} = j^*_{pass,K} + j^*_{act,K} = \left[\frac{P_KE_mF}{RT(1 - e^{-E_mF/RT})} + \frac{S_K}{C_{e,K}}\right] \cdot C^*_{e,K} + \left[\frac{P_KE_m\mathrm{Fe}^{-E_mF/RT}}{RT(1 - e^{-E_mF/RT})}\right] \cdot C^*_{i,K} \quad (17)$$

$$J_{tot,Na} = j^*_{pass,Na} + j^*_{act}\mathrm{Na} = \frac{P_{Na}E_mF}{RT(1 - e^{-E_mF/RT})} \cdot C_e^*{}_{,Na}$$

$$-\left[\frac{P_{Na}E_mFe^{-E_mF/RT}}{RT(1-e^{-E_mF/RT})}+\frac{S_{Na}}{C_{i,Na}}\right]\cdot C^*_{i,Na} \quad (18)$$

These summed fluxes are identical to the term $K_2 \cdot C_i^* - K_1 \cdot C_e^*$ of equation (1). Thus:

$$K_{1,K}=\frac{P_KE_m}{RT(1-e^{-E_mF/RT})}+\frac{S_K}{C_{e,K}} \quad (19)$$

$$K_{2,K}=\frac{P_KE_mFe^{-E_mF/RT}}{RT(1-e^{-E_mF/RT})} \quad (20)$$

$$K_{1,Na}=\frac{P_{Na}E_mF}{RT(1-e^{-E_mF/RT})} \quad (21)$$

$$K_{2,Na}=\frac{P_{Na}E_mFe^{-E_mF/RT}}{RT(1-e^{-E_mF/RT})}+\frac{S_{Na}}{C_{i,Na}} \quad (22)$$

Since the system is at steady state with respect to the total ions (tagged plus untagged)

$$K_1 \cdot C_e = K_2 \cdot C_i \text{ (for each ion)} \quad (23)$$

Combining Equations (1), (2), (17), (18), and (23)

$$C_i=\frac{K_2}{K_1}\cdot C_e \text{ (for each ion)} \quad (24)$$

$$P_K=\frac{K_{2,K}RT(1-e^{-E_mF/RT})}{E_mFe^{-E_mF/RT}} \quad (25)$$

$$P_{Na}=\frac{K_{1,Na}RT(1-e^{-E_mF/RT})}{F_mF} \quad (26)$$

$$S_K=C_{e,K}\cdot K_{1,K}\left(1-\frac{C_{e,K}}{C_{i,K}}\cdot\frac{1}{e^{-E_mF/RT}}\right) \quad (27)$$

$$S_{Na}=C_{i,Na}\cdot K_{2,Na}\left(1-\frac{C_{i,Na})}{C_{e,Na}}\cdot e^{-E_mF/RT}\right) \quad (28)$$

The experimental data, when fit to the model (Equations 1 and 2) with a parameter optimization technique, yields values for K_1 and K_2 as given in *Table 1*. Equations 24 to 28 are then used to calculate the permeability (P), active transport rate (S) and internal concentration (C_i) of the ion in question.

Ⅳ. Results

(1) Membrane Potentil

The mean value of the membrane potential was 50 mV (s.D. = ±6.4, n = 24). This is in good agreement with the Hendrickx and Casteels[10] value of 57 mV for ear artery of rabbit. Only membrane potentials stable for 20 sec or longer after the penetration were selected to calculate the mean value. The electronics used for the measurement were not fast enough to monitor the spontaneous spike activity, if any, of the preparation.

(2) Extracellular and Intracellular Volumes

The total water space was 0.760 m*l*/gm tissue (s.d. = ±.031, n = 75). The extracellular space of the tissue under tension from the rod was 0.413 m*l*/gm tissue (s.d. = ±.049, n = 21) which leaves an intracellular space of 0.347 m*l*/gm tissue.

(3) Mass Transfer Properties of the Vascular Smooth Muscle Cells for Na^+ and K^+

Fig. 3 shows the results of a typical experiment to measure the ^{22}Na and ^{42}K effluxes from the tissue preparations in normal Ringer's. It should be noted that the ^{22}Na washout rate is much faster than that for ^{42}K. This is expected from the distribution of these ions in the tissue which shows high extracellular and low intracellular concentration for the Na^+ and the opposite for the K^+. The loss of tracer ions from the tissue occurs in two stages; a fairly rapid diffusion of tracer ions from the extracellular space, being followed by a relatively slow exchange of intracellular ions. Thus most of

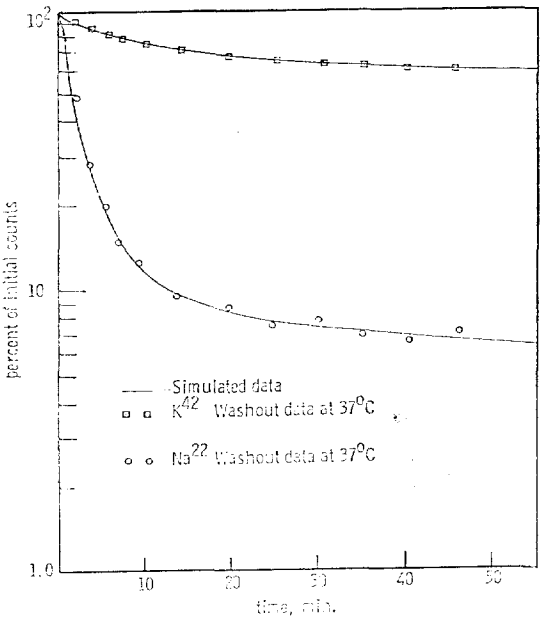


Fig. 3. Typical washout data normalized based on initial countrates for comparison

the potassium ions must overcome both the membrane and diffusional resistance to transfer while most of the sodium need only to diffuse out of the extracellular space. These two processes so closely interact with each other that it is impossible to resolve the washout curve into the two individual proce sses as had been attempted by others.[5,7,12] Instead, a parameter optimization procedure was employed to fit the entire washout data to the model (Equations 1, 2). Optimized values of parameters K_1 and K_2, were obtained from each experiment and the mean values of each parameter was calulated. The K_1 and K_2, which are lumped parameters, are functions of several membrane properties affecting ionic fluxes, including membrane potential, membrane permeability, rate of active transport, etc. (See Equations 19 to 22).

The values for K_1 and K_2 are listed in *Table 1* along with the values for other cellular porperties (P,S,C_i) calculated from the mass transfer rate constants.

The mean values of the data on the diffusion coefficient obtained through the preliminary optimizing process are also given in *Table 1*.

While there are no data to compare with our results it appears that the the values of P_{Na} and P_K are reasonable. Jones[11] reported permeabilities for sodium and potassium of P_{Na} and P_K were 1.5×10^{-8} and 7.1×10^{-8} cm/sec, respectively. However, since these values were obtained with rat aorta and were caculated based on the Goldman equation involving only passive fluxes, good agreement with our results is not expected.

The calculated rate of active transport of sodium ions is about twice the value for the K^+, indicating the active transport mechanism is electrogenic.

Reported data on the intracellular ion concentration show a wide range of variation, particulary for the sodium ions, mainly because of the uncertainties involved in quantifying the amount of bound ions and the size of the intracellular space. Since the intracellular concentrations obtained here account only for freely diffusible ions, they would be expected to belower than other values estimated from the chemical analysis. The values of 19 and 150 mM/l obtained from this study for the $C_{Na^+,i}$ and $C_{k^+,i}$, respectively, compare favorably with other investigators' results.[8,11,16] Previously peported values range from 12 to 50 mM/l for Na^+ and from 130 to 160 for K^+.[5,8,11,16]

(4) Effects of Lowering Temperature on the Sodium Washout

In order to observe temperature effects on

Table 1. Calculated Transport Parameters for Na^+ and K^+ in Canine Femoral Artery Smooth Muscle Cells

			C_i	$P(x10^8)$	$S(x10^9)$	$D(x10^6)$	$K_1(x10^8)$	$K_2(x10^8)$
			mEq/l	cm/sec	mEq/cm²sec	cm²/sec	cm/sec	cm/sec
Na^+,	37°C	(n=12)	18.7±6.4	0.6±0.2	2.1±0.68	8.33±0.73	1.45±0.48	11.80±3.0
Na^+,	21°C	(n=4)	27.5±4.1	0.5±0.08	1.5±0.26	6.88±0.48	1.08±0.18	5.9±1.57
K^+,	37°C	(n=12)	150.0±13.3	2.9±0.64	0.9±0.12	12.3±1.24	2.88±0.43	8.18±1.83

C_i = intracellular concentration of the ion.
P = permeability of the ion through the smooth muscle cell.
S = rate of active pumping of the ion.
D = diffusioncoefficient of the ion in the extracellular space.
K_1 and K_2 = mass transfer rate constants.

sodium efflux, experiments were performed at two different temperature levels, 37°C and room temperature (21±2°C). Four experiments were performed at room temperature and the results are given in *Table 1*. Normalized data for typical washout experiments at 37° and 21°C are compared in *Fig. 4*. These results suggest that the rate of ^{22}Na efflux was reduced by lowering the temperature. The dependency of diffusion processes on temperature can explain the lower values of D and P at reduced temperature. The rate of active transport of sodium ions (S) was also reduced with the decrease in the temperature. However, the intracellular sodium concentration was increased by about 50% over the control value.

It is now generally believed that the ouabain sensitive Na-K ATPase is intimately related to the active mechanism. By lowering the temperature, the activity of the enzyme is reduced and this reduced activity should be reflected as a corresponding decrease in the rate of active transport. The-reduced rate of active transport is, then, expected to increase $C_{Na^+,i}$. Thus, the results of this work for low temperature subtantiate the current understanding of the behavior of the Na-K pump.

V. Disucussion

In view of the relationship between changes in the cell potential and the contracile process of vascular smooth muscle, it appears that any factor which affects the membrane potential (ionic composition, membrane permeability, active ionic flux, or any combination of these) may play a very important

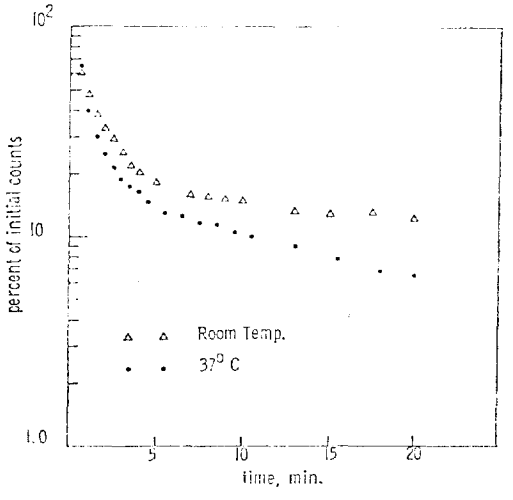


Fig. 4. Comparison of the normalized washout curves for Na^+ obtained with tissues both incubated and washed in normal Ringer's at 37°C and room temperature, respectively.

role in contraction.

In order to more precisely elucidate the roles of Na^+ and K^+ in vascular smooth muscle contraction, the exchange of sodium and potassium ions between the muscle cells and the surronding media have been studied by many investigators.[4,5,7,10] In those studies, the washout data have usually been fitted to equations involving sums of exponentials, based on compartmental analysis. The washout data are then interpreted in terms of the factors obtained from the washout data using graphical methods. In general, more than two exponentials are required to fit the washout data, suggesting that tissues being studied consisted of at least three compartments.[5,7,15] With the present anatomical knowledge, biological identification of the compartments implicated in the analysis of the washout data becomes a considerable problem. Furthermore, the basic problem as to how many exponentials are necessary to fit a set of washout data remains to be resolved, and in any case has little physiolcgical significance.

An approach similar to the one used here was taken by other investigators for the analysis of washout data. Harris and Burn[9] used essentially the same model for the study of ionic fluxes in frog satorius muscle but the data were analyzed by an approximate graphical technique and active pumping was not considered. Jones and Karreman[12] used a numerical technique with a computer to analyze equivalent of Equations 1 and 2 and presented data for the exchange of sodium ions in canine carotid arteries. Based on a concept quite different from the compartmental model, they optimized the diffusion coefficient in the extracellular space. However, the values of the rate constants, K_1 and K_2 were obtained with the graphical method which is based on the compartmental model. Thus none of the previous investigators have used a model which (a) considered diffusion in the extracellular space, (b) interpreted the membrane transfer coefficients in terms of both passive and active fluxes, and (c) used optimization techniques to fit the data to the model. As a result, the methods presented here appear to be a significant improvement in the interpretation of tissue washout data.

The electrogenic nature of the active transport of sodium and potassium ions as well as the effects of lowering temperature on the sodium washout is consistent with the hypothesis that an electrogenic pump plays an important role in maintaining the cell potential and in regulation of vascular resistance to blood flow. Furthermore, in a series of preliminary experiments we also observed a decrease in the washout rate of ^{22}Na, on lowering the external potassium level to 2 m Eq/l. Even though statistical significance could not be shown, the mean value for the rate of active transport of sodium ions was 13% less than than the control value, while there was no change in permeabillity. From this we may speculate that the changes in potential are not mediated through changes in permeability when $(K^+)_e$ is altered.

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