Br. J. Pharmac. (1986), **88**, 881–887

The electrogenic Na-pump and spontaneous contraction of the hypokalemic rat duodenum

N. Akaike[1] & Y. Wakita*

Department of Physiology, Faculty of Medicine, Kyushu University 60, Fukuoka 812 and Department of Physiology*, Kumamoto University Medical School, Kumamoto 860, Japan

1 The effects of the electrogenic Na-pump on spontaneous contraction in the isolated, longitudinal muscle of the duodenum of rats which had been on a potassium-deficient diet for 7 weeks, have been investigated. Intracellular levels of Na^+ are increased by this diet.

2 The spontaneous contraction of the duodenal muscle was stopped, transiently, by 0.5 to 120 mM-K^+ Krebs solution. The period of decrease of tone and amplitude occurring immediately after adding K^+ was shortened when the external K^+ concentration ($[K]_o$) was increased from 0.5 to 120 mM.

3 The decrease in tone and amplitude induced by K^+ was abolished by exposure of the tissue to 0 mM $[K]_o$, by exposure to a temperature below 14°C, and in the presence of ouabain (3×10^{-5}–10^{-4} M).

4 The spontaneous contraction of 'Na-rich' duodenum in bathing medium containing 15 mM K^+ and following inhibition of the electrogenic Na-pump with cooling or ouabain was much the same as in the duodenum from rats fed balanced diets: i.e., increase of contractile tone immediately after adding K^+.

5 To activate the Na-pump in 'Na-rich' duodenum, the external K^+ could be replaced by Rb^+, Cs^+, NH_4^+ and Tl^{3+}. The effectiveness was in the order $K^+ > Rb^+ > Cs^+ > NH_4^+ > Tl^{3+}$.

6 The possible existence of a neuronal or hormonal inhibitory mechanism affecting the active Na-K transport in rat smooth muscle *in situ*, under conditions of hypokalemia, is discussed.

Introduction

When rats are fed a potassium-deficient diet for a few weeks a considerable decrease in intracellular K^+ concentrations ($[K]_i$) and an increase of intracellular Na^+ concentrations ($[Na]_i$) results. The magnitude of the effect decreases in different muscles in the order of: skeletal > intestinal (duodenum) > heart (Conway & Hingerty, 1948; Muntwyler & Griffin, 1951; Cooke *et al.*, 1952; Bradbury & Kleeman, 1967; Johanson *et al.*, 1974; Nattie, 1977; Akaike, 1981; Akaike *et al.*, 1983). When 'Na-rich' skeletal muscles such as the soleus and extensor digitorum longus from hypokalemic rats were soaked in a Krebs solution containing more than 1.5 mM K^+ at 37°C, $[Na]_i$ was promptly reduced whereas $[K]_i$ was increased. At the start of the recovery, the 'Na-rich' skeletal muscle fibres were hyperpolarized (Akaike, 1974; 1975a; 1976; 1981). The period of hyperpolarization was shortened with increasing levels of $[K]_o$. The K^+-sensitive hyperpolarization was abolished in K^+-free solution, on cooling to ca. 4°C, and in the presence of 10^{-4} M ouabain (Akaike, 1974; 1975a). However, the contribution of the electrogenic Na-pump to contractile activities of 'Na-rich' smooth muscles isolated from hypokalemic rats has remained unknown. The present study attempts to determine the manner in which the contractile activities of the duodenum from hypokalemic rats differ from those of normal rats in the presence of 0.5 to 120 mM external-K^+, and to observe the effects of K^+-free solutions, low temperatures and exposure to ouabain upon them.

Methods

Male Wistar rats weighing between 200 and 280 g were kept for 7 weeks under conditions of a 55 to 60% humidity at about 22°C and were given free access to a normal rat cake diet (control group) or to a potassium-deficient diet (hypokalemic group). At the start of experiments, rats were stunned and bled, the duodenum (2 cm caudal to the pylorus) was isolated and emptied of contents by flushing with 0.9% saline, and longitudinal segments (1 cm) were prepared. The

[1]Author for correspondence.

preparations were suspended in an organ bath filled with 15 ml Krebs solution without K^+ at 37°C and superfused with the solution through a heating coil, at a rate of 2 ml min^{-1}. A rapid change in bathing solution from K^+-free to K^+-rich Krebs solution, with or without ouabain, was achieved by flushing through 30 ml of the new test solution within 30 s. The preparation was cooled to 5°C in 30 s or less by application of a solution through tubing coiled in an ice bath containing salt. The temperature was monitored by means of a thermistor and was kept constant within ±1°C during the experiments.

Longitudinal smooth muscle contraction measurement

Isometric contraction of the duodenum was recorded on an inkwriting oscillograph through a strain gauge transducer. The initial tension applied to the preparation was adjusted to about 1 g, since the tone and amplitude of spontaneous contraction are related to the initial tension.

Solutions

Modified Krebs solution was used of the following

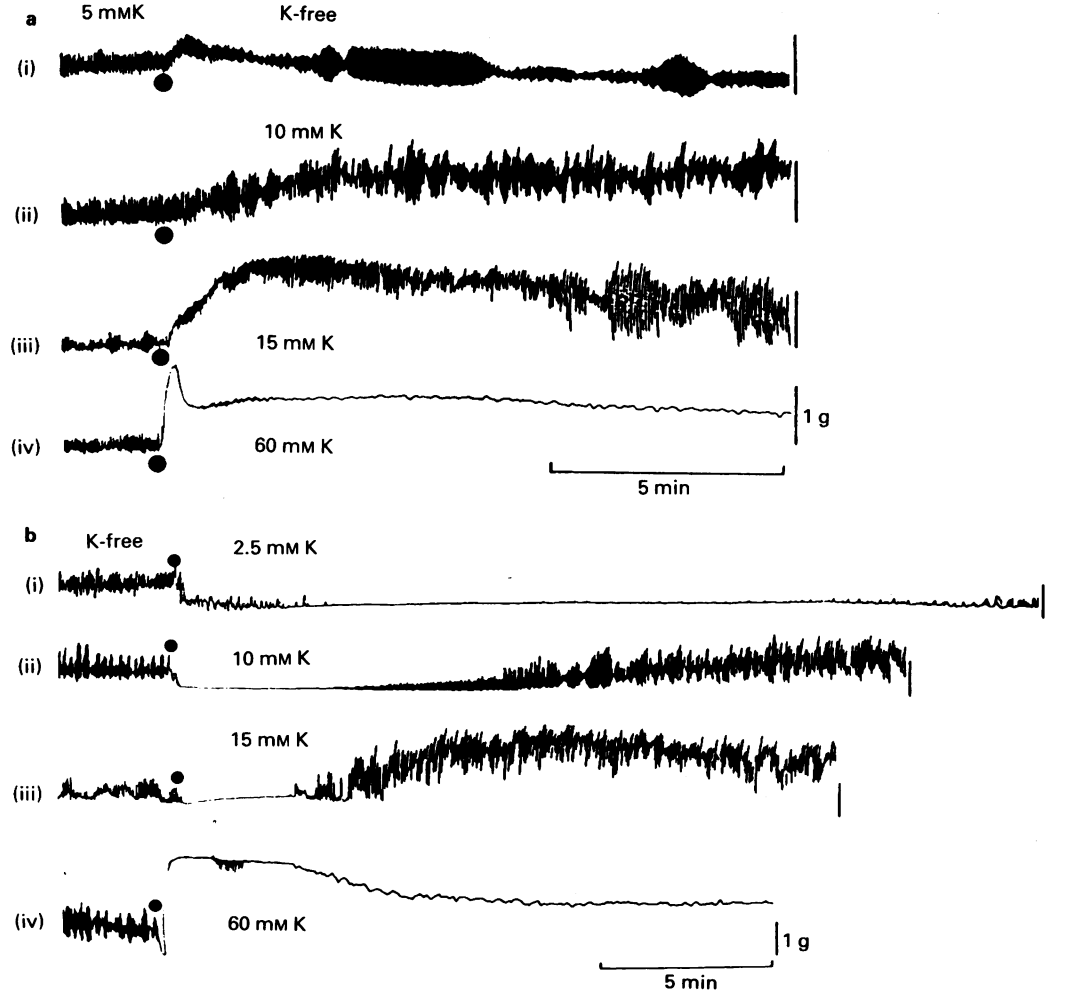

Figure 1 Effects of external K^+ on spontaneous contraction of duodenal strips from normal and hypokalemic rats. (a) Recordings (i) to (iv) were obtained from different preparations of normal rats. Note a decrease of tone in K^+-free medium and an increase of tone with raising external K^+. (b) All recordings were made from different preparations of hypokalemic rats. Note a transient decrease in tone and amplitude of spontaneous contraction after the application of K^+, the duration of which is shortened by increasing external K^+ concentrations.

composition (mM): NaCl 115, KCl 5, Ca-gluconate 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.2, Na_2HPO_4 0.4, $NaHCO_3$ 28 and glucose 11. All solutions were prepared with distilled and deionized water from tenfold concentrated stock solutions of the major ions. Glucose, Ca-gluconate, NaH_2PO_4, Na_2HPO_4 and $NaHCO_3$ were added to the solution before each experiment. All solutions were equilibrated with 95% O_2 and 5% CO_2 throughout the experiments and the pH of solutions was 7.3 to 7.4. Modified Krebs solutions containing various concentrations of K^+ were prepared by replacing NaCl by an equimolar amount of KCl or by replacing KCl with NaCl.

Muscle Na^+ and K^+ contents

Na^+ and K^+ contents (mmol kg^{-1} H_2O) in the duodenum were measured by means of flame photometry (Desmedt, 1953; Akaike, 1975a).

Statistical methods

The data were analysed statistically by Student's *t* test. The numerical values are given as mean ± s.d.

Results

Effects of external K^+ concentration on spontaneous contraction of the duodenum

Figure 1a shows the time course of changes in tone and amplitude of the spontaneous contraction of the duodenum from normal rats, in solutions containing 0, 5, 10, 15, 60 and 120 mM-K^+. In K^+-free medium, the tone decreased gradually by -0.4 ± 0.08 g (6 preparations). Upon adding 10 and 15 mM-K^+ solution, both the tone and amplitude of the spontaneous contraction increased. Further increase in $[K]_o$ to 60 and 120 mM led to a rapid increase in the tone, which was earlier and more short-lived in higher than in lower $[K]_o$.

Exposure of the duodenum from hypokalemic rats to solutions containing 0.5 to 120 mM-K^+ induced a transient decrease in tone and a disappearance of the spontaneous contractions (Figure 1b). The periods of decreased tone after adding K^+ became shorter with increasing $[K]_o$. The duration is summarized in Figure 2a.

Figure 2b shows the time course of changes of Na^+ and K^+ content of 'Na-rich' duodenum when immersed in 15 mM-K^+ solution. The Na^+ and K^+ contents of the muscle are presented as mmol kg^{-1} H_2O of muscle wet weight, since we do not know the exact extracellular space in the tissue. After exposure to recovery solution containing 145 mM-Na^+ and 15 mM-K^+ for 20 min at 37°C, the Na^+ of the 'Na-rich' muscles was reduced to 56.6 ± 1.8 from 72.3 ± 2.8 mmol kg^{-1} H_2O ($n = 15$) and K^+ was increased to 77.3 ± 2.2 from 55.2 ± 2.0 mmol kg^{-1} H_2O ($n = 12$), these being close to the values in normal rats (Na^+, 51.6 ± 1.6 and K^+, 81.2 ± 2.0 mmol kg^{-1} H_2O, $n = 10$). However, Na^+ or K^+ recovery was incomplete since the contents of these ions in recovered smooth muscles differed significantly from those in normal rats.

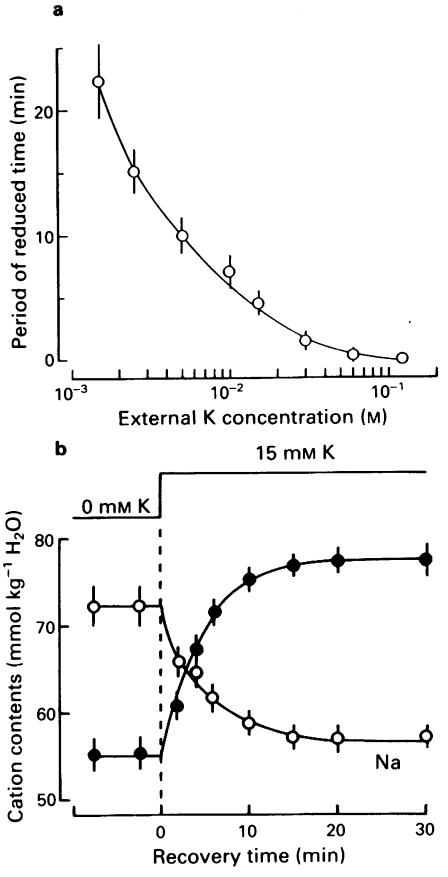

Figure 2 (a) Change in the period of reduced tone in duodenum of hypokalemic rats induced by various external K^+ concentrations. Each point represents the average value of 8 preparations; vertical bars show ± 1 s.d. (b) Na^+ and K^+ content of duodenum from hypokalemic rats during recovery at 37°C. At zero time Krebs solution containing 15 mM-K^+ was added. Each point is the average of 12 preparations; vertical bars indicate ± 1 s.d. Note the rapid recovery in tissue cation contents after adding K^+.

Effects of K^+-like cations on spontaneous contraction of the 'Na-rich' duodenum

In Krebs solution containing 5 mM K^+, Rb^+ or Cs^+, there were rapid decreases in tone and the rate of decrease was greater in the order $K^+ > Rb^+ > Cs^+$ ($n = 5$). A cessation of the spontaneous contraction was produced by 5 mM-K^+ while spontaneous contractions still occurred in the presence of Rb^+ or Cs^+ (Figure 3). Furthermore, adding 5 mM NH_4^+ to K^+-free Krebs solution caused not only a transient decrease of tone, but also a prolonged decrease in the contractile frequency in which the amplitude of spontaneous contractions was augmented with time. In the presence of 0.5 mM Tl^{3+}, the tone decreased for a long period and the spontaneous contractions were infrequent and of reduced amplitude.

Effect of ouabain

Ouabain is a specific inhibitor of Na^+-K^+-dependent ATPase (Skou, 1965). Figure 4a shows changes in tone and in the amplitude of spontaneous contractions of 'Na-rich' duodenum, in response to 15 mM-K^+ and in the presence or absence of various concentrations of ouabain at 37°C. The addition of 10^{-4} M ouabain immediately reversed the relaxation caused by 15 mM-K^+ Krebs solution and the effect on contractile activity was dose-dependent. Figure 4b shows the relationship between the period of reduced tone and ouabain concentration. The period of reduced tone decreased with increasing ouabain concentration. This decrease in tone after the addition of K^+ is probably due to membrane hyperpolarization resulting from activation of the K^+-sensitive electrogenic Na-pump.

Effect of cooling

The effects of adding 15 mM-K^+ to the K^+-free Krebs solution bathing 'Na-rich' duodenum at various temperatures (5 to 37°C) are shown in Figure 5a. When K^+ was added to the solution at temperatures between 15 and 37°C, the decrease in the contractile tone occurred more slowly at lower temperatures. The

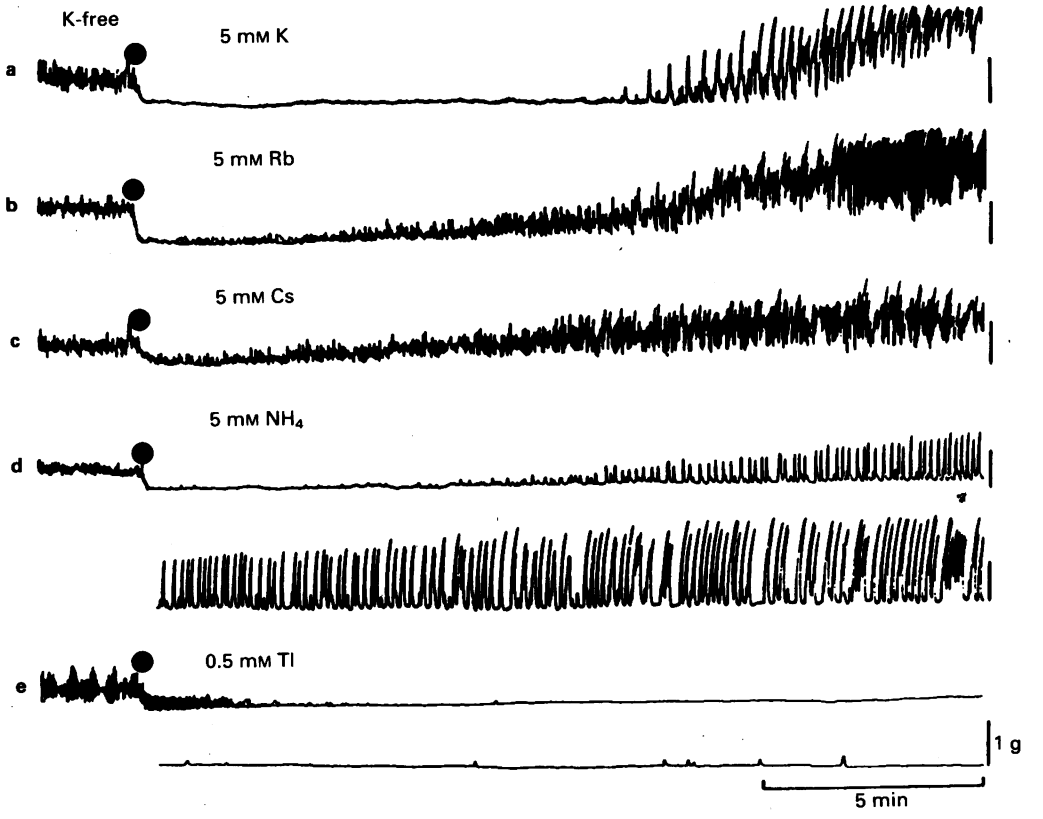

Figure 3 Spontaneous contraction of the hypokalemic rat duodenum during recovery in Krebs solution containing 5 mM K^+, Rb^+, Cs^+ and NH_4^+ and 0.5 mM Tl^{3+} at 37°C. Each recording was made from a different preparation.

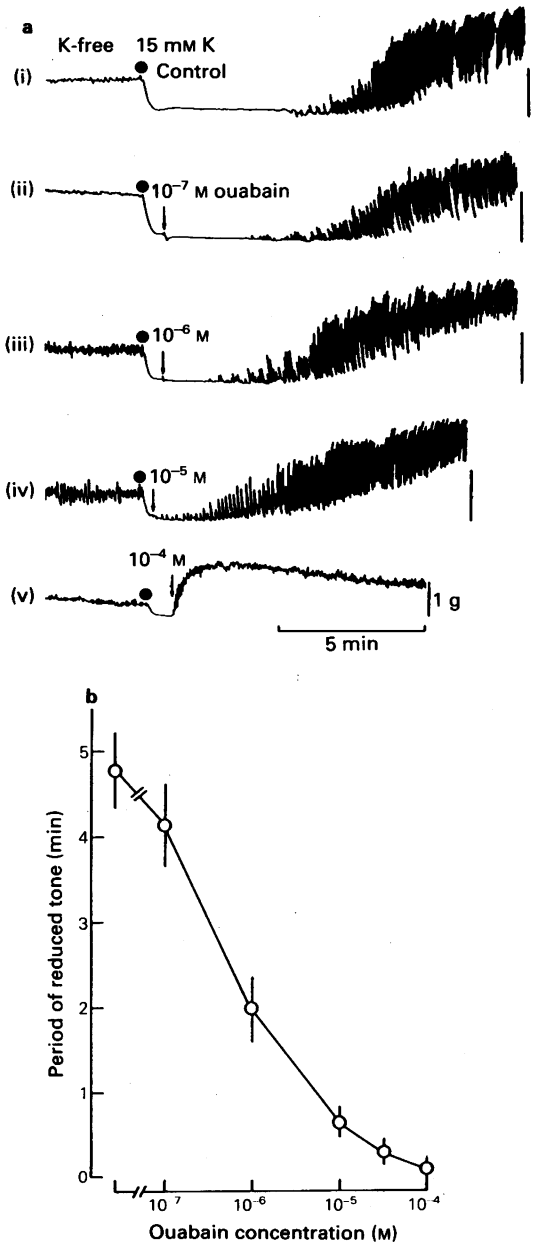

Figure 4 (a) Changes in spontaneous contractions during immersion of 'Na-rich' duodenum in Krebs solution containing 15 mM-K^+ in the presence or absence of 10^{-7} to 10^{-4} M ouabain. Recordings were obtained from different preparations. (b) Relationship between period of reduced tone of 'Na-rich' duodenum and ouabain concentration. Each point represents an average value of 8 experiments, vertical bars show ± 1 s.d.

gradual increase in tone during K^+ re-accumulation also became slower at lower temperatures. However, the addition of K^+ immediately increased the contractile tone at temperatures below 14°C (Figure 5a (v) and (vi)), thereby suggesting blockade of the electrogenic Na-pump by cooling. Figure 5b summarizes the effects of temperature on the peak tone (shown by the arrow on each trace of Figure 5a), in the presence of 15 mM-K^+.

Discussion

The addition of 0.5 to 30 mM-K^+ to K^+-free Krebs solution bathing 'Na-rich' skeletal muscles isolated from hypokalemic rats transiently hyperpolarized the resting membrane potential beyond the potential predicted from the Goldman equation or even beyond the K^+ equilibrium potential (E_K). The hyperpolarization declined progressively during immersion in a recovery solution containing K^+, and the measured membrane potential became equal to E_K, as a considerable Na^+ extrusion and K^+ uptake occurred (Akaike, 1975a). Moreover, the K^+-sensitive hyperpolarization was abolished on exposure to 0 mM-K^+, on cooling to ca. 4°C, and in the presence of ouabain (10^{-4} M), a compound which inhibits the electrogenic Na-pump. In the present experiments, there was a marked transient decrease in the tone after adding 0.5 to 120 mM-K^+ to K^+-free Krebs solution bathing the 'Na-rich' duodenum isolated from hypokalemic rats. Such a decrease in the contractile tone was abruptly abolished by lowering the temperature to below 14°C, or by 3×10^{-5} to 10^{-4} M ouabain. This would suggest that reduction in the tone is due to a membrane hyperpolarization by activation of the electrogenic Na-pump.

In other experiments, we found that increasing the K^+ concentration in the recovery Krebs solution led to a hyperpolarization of 'Na-rich' skeletal muscle fibres from hypokalemic rats which appeared earlier and the duration of which was shortened with increasing concentration (Akaike, 1975a). The peak post-tetanic hyperpolarization of non-myelinated nerve fibres of the rabbit was also found to decline significantly at a $[K]_o$ exceeding 2 mM (Rang & Ritchie, 1968), and the peak hyperpolarization of 'Na-loaded' taenia coli of guinea-pig diminished and disappeared more rapidly with increasing $[K]_o$ (Casteels *et al.*, 1971). These results are consistent with the present findings, in which the period of reduced tone of 'Na-rich' duodenum with the addition of K^+ became shorter when increasing $[K]_o$ from 0.5 to 120 mM.

In the present experiments, the addition of 5 mM K^+, Rb^+, Cs^+, NH_4^+ or 0.5 mM Tl^{3+} to K^+-free solution bathing 'Na-rich' duodenum resulted in a decrease or cessation of tone and the number of

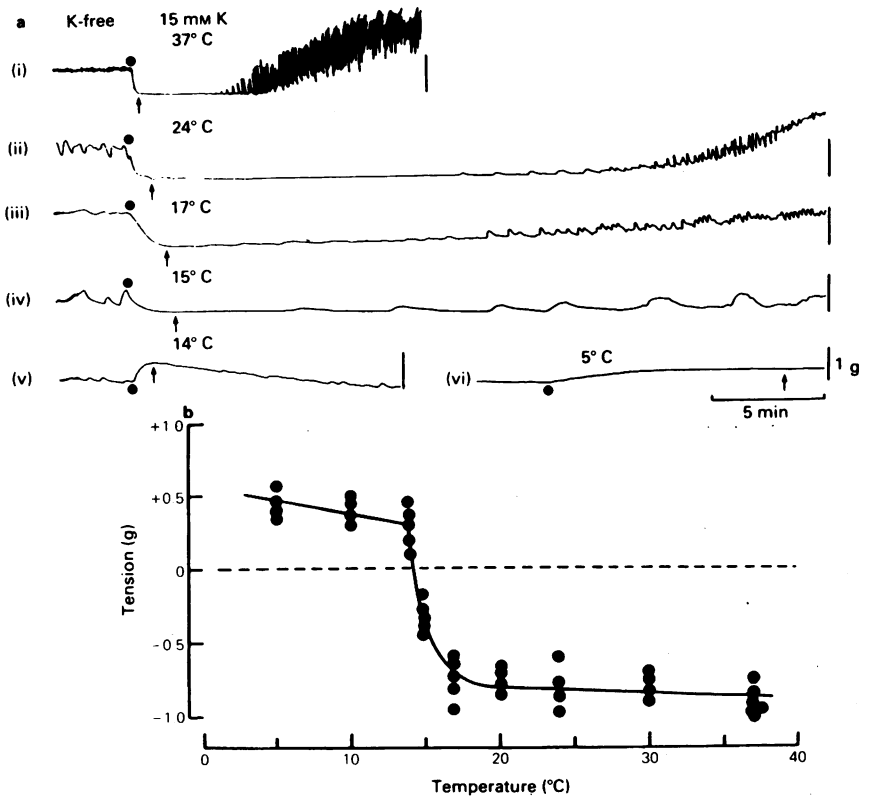

Figure 5 Effects of temperature on the spontaneous contraction of the hypokalemic rat duodenum. (a) Effect of cooling on reduction of tone caused by adding 15 mM-K^+ to K^+-free medium. All recordings were from different preparations and measurements at peak tone are indicated by an arrow. (b) Relation between contractile tension of 'Na-rich' duodenum after adding 15 mM-K^+ Krebs solution and temperature. The dashed line represents the tone in K^+-free medium. Each point was obtained from individual preparations. Note a marked change of tone between 14 and 17°C.

spontaneous contractions was reduced. This would suggest that the electrogenic Na-pump in the duodenal muscle is activated not only by K^+ but also by Rb^+, Cs^+, NH_4^+ and Tl^{3+}. Rb^+, Cs^+, NH_4^+ and Tl^{3+} can also substitute for K^+ in increasing the rate of oxygen uptake associated with Na-pumping in crab nerve (Barker & Connelly, 1966). The order of effectiveness of equimolar concentrations of K^+, Rb^+, Cs^+ and NH_4^+ in stimulating the electrogenic Na-pump in 'Na-rich' skeletal muscles of hypokalemic rats was $Rb^+ > K^+ > NH_4^+ > Cs^+$ (Akaike, 1975b). In the 'Na-rich' duodenum, the effectiveness was in the order $K^+ > Rb^+ > Cs^+ > NH_4^+ > Tl^{3+}$. This order is similar to the relative order of cations penetrating various cell membranes, such as frog muscle (Conway & Moore, 1945; Bolingbroke *et al.*, 1961), barnacle and lobster muscle fibres (Hagiwara *et al.*, 1964; Gainer & Grundfest, 1968), frog and rabbit corneal basal cells (Akaike & Hori, 1970; Akaike, 1971) and frog crystalline lens fibres (Murata *et al.*, 1984). In addition, the prolonged suppression of tone of 'Na-rich' duodenum in Krebs solution containing NH_4^+ or Tl^{3+} may be attributed to the high activity of the electrogenic Na-pump found in crab nerve (Barker & Connelly, 1966) and in 'Na-rich' skeletal muscle (Akaike, 1975b).

In rats fed a potassium-deficient diet for 5 to 7 weeks, the plasma K^+ level was 1.6 to 2 mM, a value sufficiently high to stimulate the electrogenic Na-pump in skeletal muscle. Thus, in skeletal muscles isolated from hypokalemic rats 1.5 mM-K^+ Krebs solution evokes a reduction in $[Na]_i$ while $[K]_i$ is increased. The high $[Na]_i$ and low $[K]_i$ in skeletal muscles *in vivo* are not a direct result of pump inhibition by plasma hypokalemia. In fact, peripheral nerve section, cervical and brain-stem transection, decerebration, cortical spreading depression with 20% KCl, and electrolytic lesioning of the ventromedial hypothalamic nucleus (VMH) activate Na^+ and K^+ transport in skeletal muscles *in vivo* during hypok-

alemia, thereby indicating that the CNS directly imposes an inhibition on active transport (Akaike, 1974; 1975a; 1976; 1979; 1981; 1982; Akaike *et al.*, 1983; 1986; Yoshimatsu *et al.*, 1986). The spontaneous contraction of the 'Na-rich' duodenum of hypokalemic rats was also inhibited by adding 1.5 mM K^+ to K^+-free Krebs solution. All these observations indicate that the high $[Na]_i$ and low $[K]_i$ in the duodenum *in situ* of hypokalemic rats may not be due to pump inhibition by plasma hypokalemia; rather a neuronal or humoral regulation which suppresses the active Na-pump in smooth muscles of hypokalemic rats has to be considered.

We are grateful to M. Ohara for valuable comments on the manuscript. The present study was supported by a Grant to N.A. from the Muscular Dystrophy Association, U.S.A. and Grants-in-Aid 59225018 from the Ministry of Education, Sciences and Culture, Japan.

References

AKAIKE, N. (1971). The origin of the basal cell potential in frog corneal epithelium. *J. Physiol. Lond.*, **219**, 57–75.

AKAIKE, N. (1974). Operation of an electrogenic Na pump in mammalian red muscle fibre. *Life Sci.*, **14**, 141–147.

AKAIKE, N. (1975a). Contribution of an electrogenic sodium pump to membrane potential in mammalian skeletal muscle fibres. *J. Physiol. Lond.*, **245**, 499–520.

AKAIKE, N. (1975b). Activation of electrogenic sodium pump in mammalian skeletal muscle by external cations. *Pflügers Arch.*, **355**, 281–290.

AKAIKE, N. (1976). Intracellular ion concentration and electrical activity in potassium-depleted mammalian soleus muscle fibers. *Pflügers Arch.*, **362**, 15–20.

AKAIKE, N. (1979). CNS effects on muscle Na/K levels in hypokalemia. *Brain Res.*, **178**, 175–178.

AKAIKE, N. (1981). Sodium pump in skeletal muscle: central nervous system-induced suppression by α-adrenoreceptors. *Science, N.Y.*, **213**, 1252–1254.

AKAIKE, N. (1982). Hyperpolarization of mammalian skeletal muscle fibers in K-free media. *Am. J. Physiol.*, **242**, 12–18.

AKAIKE, N. & HORI, M. (1970). Effects of anions and cations on membrane potential of rabbit corneal epithelium. *Am. J. Physiol.*, **219**, 1811–1818.

AKAIKE, N., HIRATA, A., KIYOHARA, T. & OYAMA, Y. (1983). Neural regulation on the active sodium-potassium transport in hypokalemic rat skeletal muscles. *J. Physiol. Lond.*, **341**, 245–255.

AKAIKE, N., OOMURA, Y., YOSHIMATSU, H. & KATAFUCHI, T. (1986). Na-pump regulation by autonomic nervous system in hypokalemic rat skeletal muscle. In *Emotions: Neuronal and Humoral Control.* ed. Oomura, Y. pp. 431–439. Tokyo: Japan Scientific Societies Press.

BARKER, P.F. & CONNELLY, C.M. (1966). Some properties of the external activation site of the Na pump in crab nerve. *J. Physiol. Lond.*, **185**, 270–297.

BOLINGBROKE, V., HARRIS, E.J. & SJODIN, R.A. (1961). Rubidium and caesium entry and cation interaction in frog skeletal muscle. *J. Physiol. Lond.*, **157**, 289–305.

BRADBURY, N.W.B. & KLEEMAN, C.R. (1967). Stability of the potassium content of cerebrospinal fluid and brain. *Am. J. Physiol.*, **213**, 519–528.

CASTEELS, R., DROOGMANS, G. & HENDRICK, H. (1971). Membrane potential of smooth muscle cells in K-free solution. *J. Physiol. Lond.*, **217**, 281–295.

CONWAY, E.J. & HINGERTY, D. (1948). Relations between potassium and sodium levels in mammalian muscle and blood plasma. *Biochem. J.*, **42**, 372–376.

CONWAY, E.J & MOORE, P.T. (1945). Cation and anion permeability constants for the muscle fibre membrane. *Nature, Lond.*, **156**, 170–171.

COOKE, R.E., SEGAR, W.E., CHEEK, D.B., COVILLE, F.E. & DARROW, D.C. (1952). The extrarenal correction of alkalosis associated with potassium deficiency. *J. clin. Invest.*, **31**, 798–805.

DESMEDT, J.E. (1953). Electrical activity and intracellular sodium concentration in frog muscle. *J. Physiol. Lond.*, **121**, 191–205.

GAINER, H. & GRUNDFEST, H. (1968). Permeability of alkali metal cations in lobster muscle, a comparison of electrophysiological and osmometric analyses. *J. gen. Physiol.*, **51**, 399–425.

HAGIWARA, S., CHICHIBU, S. & NAKA, K. (1964). The effects of various ions on resting and spike potentials of barnacle muscle fibres. *J. gen. Physiol.*, **48**, 163–179.

JOHANSON, C.E., REED, D.J. & WOODBURY, D.M. (1974). Active transport of sodium and potassium by the choroid plexus of the rat. *J. Physiol. Lond.*, **241**, 359–372.

MUNTWYLER, E. & GRIFFIN, G.E. (1951). Effect of potassium on electrolytes of rat plasma and muscle. *J. biol. Chem.*, **193**, 563–573.

MURATA, T., OKAJIMA, Y. & AKAIKE, N. (1984). Origins of the transient anterior-posterior asymmetry in the frog lens fiber potential. *Comp. Biochem. Physiol.*, **77A**, 287–291.

NATTIE, E.E. (1977). Brain tissue potassium in normal and potassium depleted rats. *Life Sci., Oxford*, **21**, 1851–1856.

RANG, H.P. & RITCHIE, J.M. (1968). On the electrogenic sodium pump in mammalian non-myelinated nerve fibres and its activation by various external cations. *J. Physiol. Lond.*, **196**, 183–221.

SKOU, J.C. (1965). Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. *Physiol. Rev.*, **45**, 596–617.

YOSHIMATSU, H., OOMURA, Y., KATAFUCHI, T. & AKAIKE, N. (1986). Hypothalamic regulation of sodium-potassium pump activity in skeletal muscle. *Brain Res.*, (in press).

(Received January 20, 1986.
Revised April 7, 1986.
Accepted April 12, 1986.)