European Journal of Pharmacology, 55 (1979) 281–292
Elsevier/North-Holland Biomedical Press

EFFECTS OF MONOVALENT CATIONS ON ($Na^+ + K^+$)-ATPase IN RAT BRAIN SLICES

TAI AKERA, RICHARD H. GUBITZ, THEODORE M. BRODY and THOMAS TOBIN *

*Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan 48824, U.S.A. and * Department of Veterinary Science, University of Kentucky, Lexington, Kentucky 40506, U.S.A.*

Received 11 July 1978; revised MS received 8 November 1978, accepted 25 January 1979

T. AKERA, R.H. GUBITZ, T.M. BRODY and T. TOBIN, *Effects of monovalent cations on ($Na^+ + K^+$)-ATPase in rat brain slices*, Europe. J. Pharmacol. 55 (1979) 281–292.

The influence of monovalent cations on membrane ($Na^+ + K^+$)-ATPase was estimated in vitro in intact cells from the oxygen consumption of rat brain cortical slices. High concentrations of K^+, Rb^+ or Cs^+ stimulated the respiration in the presence of Na^+. This stimulation was antagonized by ouabain in a concentration- and time-dependent manner. Additionally, only combinations of monovalent cations, that stimulate ($Na^+ + K^+$)-ATPase, increased oxygen consumption, indicating that the stimulated portion of respiration is related to the ($Na^+ + K^+$)-ATPase activity. Low concentrations of Rb^+ and Cs^+, however, failed to affect oxygen consumption. Li^+ slightly and transiently stimulated oxygen uptake at low concentrations and inhibited it at higher concentrations. Low concentrations of Tl^+ also stimulated respiration in a K^+-free medium. However, the inhibitory effects of Tl^+ were predominant at higher concentrations or in the presence of K^+. Thus, monovalent cations can alter ($Na^+ + K^+$)-ATPase activity. While Rb^+ and Li^+ produce opposite effects on this enzyme system under certain conditions, these actions do not seem to be related to the antidepressant action of Rb^+ and the antimanic action of Li^+.

Rubidium Lithium Na^+,K^+-ATPase Tissue respiration

1. Introduction

Several investigators suggested that the antidepressive action of Rb^+ and the antimanic action of Li^+ involve membrane Na^+,K^+-ATPase, or its functional correlate, the cell membrane sodium pump (Meltzer et al., 1969; Glen and Reading, 1973; Tobin et al., 1974; Dick et al., 1974; Ploeger, 1974a; Hesketh, 1976; Mendels et al., 1976). This hypothesis is primarily based upon the observation that sodium transport is altered in affective disorders (Gle et al., 1968; Naylor et al., 1970, 1973; Mendels and Frazer, 1974; Hokin-Neaverson et al., 1974; Naylor et al., 1976; Hesketh et al., 1977), and that the enzyme system is sensitive to these cations (Skou, 1960; Whittam and Ager, 1964; King et al., 1969; Willis and Fang, 1970; Post et al., 1972; Gutman et al., 1973a, b; Fieve et al., 1973; Ploeger, 1974a, b; Tobin et al., 1974; McNulty et al., 1975). Corresponding to their pharmacological actions, Rb^+ and Li^+ have been reported to exert opposite effects on this enzyme system under several experimental conditions (Post et al., 1972; Tobin et al., 1974; Han et al., 1976).

The above studies indicating that the Na^+,K^+-ATPase system can discriminate between Rb^+ and Li^+, however, were performed using isolated enzyme preparations in which the asymmetric features of the enzyme as it occurs in intact cells cannot be reproduced. It is reasonable to assume that Rb^+ and Li^+ might affect Na^+,K^+-ATPase by either substituting for or antagonizing, the natural activators of the enzyme, namely Na^+ or K^+. In intact cells, $[Na^+]_i$ and $[K^+]_o$ activate the enzyme, whereas $[Na^+]_o$ and $[K^+]_i$ antagonize the activation of the enzyme by $[K^+]_o$ and $[Na^+]_i$, respectively (Skou, 1960). Since the action of Rb^+ or Li^+ was not compared in

intact brain cells, it cannot be assumed that these cations affect Na^+,K^+-ATPase in vivo in a manner similar to that described in an isolated enzyme system. In addition, several investigators (Hokin-Neaverson et al., 1974; Hesketh et al., 1977; Frazer et al., 1978) reported that Li^+ does not alter Na^+,K^+-ATPase activity under certain conditions. Thus, the present study was undertaken to clarify the actions of Rb^+ and Li^+ in rat brain slices.

The direct measurement of the rate of ATP hydrolysis in intact cells is not possible. Attempts to estimate Na^+,K^+-ATPase activity from the amount of ATP remaining in brain tissue is complicated by a number of factors and has failed to yield definitive data (King et al., 1969). In a recent paper (Gubitz et al., 1977), however, we demonstrated that Na^+,K^+-ATPase activity in brain slices can be estimated accurately from that portion of the brain slice respiration stimulated by Na^+ in the presence of a high concentration of K^+. It was shown that this fraction of the respiration corresponds to the Na^+,K^+-stimulated ATPase activity. In the present study, the effects of monovalent cations on Na^+,K^+-ATPase activity were estimated in brain slices using this technique. The purpose of the study was to determine whether these cations affect Na^+,K^+-ATPase in intact cells in a similar manner as that observed in isolated enzyme preparations, and whether their effects on the enzyme system might account for the well-known clinical actions of these cations.

2. Materials and methods

Male Sprague Dawley rats weighing 200—250 g were used. The animals were decapitated and thin slices (approximately 0.5 mm thick) were prepared from the cerebral cortices. These were immediately weighed and incubated at 37°C for 20 min in a 100% oxygen atmosphere in 1.8 ml of an incubation medium containing (mM) 128 NaCl, 3 KCl, 1.23 $MgSO_4$, 15 sodium phosphate buffer (pH 7.4) and 24 glucose, unless otherwise indicated. Oxygen uptake was assayed manometrically by the method of Umbreit (1972) using a Gilson differential respirometer (Gilson Medical Electronics, Inc., Middleton, Wisc.). The control rate of brain slice respiration was estimated for a 30-min period following a 20-min equilibration period. Subsequently, a 0.2 ml solution containing the agent to be tested was added from the side arm of the reaction vessel to the incubation medium and oxygen consumption was assayed for four additional 30-min periods. The results were expressed as μmoles of oxygen consumed per gram tissue (wet weight) per 30 min.

The effects of monovalent cations in the presence of a high K^+ concentration were studied using a medium containing (mM) 100 KCl, 1.23 $MgSO_4$, 15 Tris phosphate buffer (pH 7.4) and 24 glucose. After the equilibration period, the chloride salt of each cation under study was added. Choline chloride was added in control vessels. Under these conditions the portion of the slice respiration stimulated by 10—100 mM Na^+ has been shown to be related to the enhanced ADP generation due to Na^+,K^+-ATPase activity (Gubitz et al., 1977).

The actions of monovalent cations on the maximally activated respiration of brain homogenates (state 3 respiration) were studied as described by Potter (1972). Briefly, thin tissue slices were prepared from the cerebral cortex as described above and then homogenized in 4 volumes of a 0.32 M sucrose solution using a Potter—Elvehjem homogenizer with a Teflon pestle driven at 800 r.p.m. All solutions and homogenates were kept at 0—2°C until incubation. A 0.25 ml aliquot of a 20% homogenate was added to 1.75 ml of an incubation medium yielding final concentrations of 2 mM Tris · ADP, 3 mM $MgCl_2$, 10 mM KH_2PO_4 buffer (adjusted to pH 7.2 with KOH), 10 mM glucose, 0.05 mM K_2 · EDTA, 0.2 mM NAD, 40 mM nicotinamide and 40 μg of hexokinase enzyme (activity, 18.5 units/mg protein).

1956). The concentration of LiCl, RbCl or CsCl was varied from 0 to 100 mM. Choline chloride was used as an osmotic substitute to maintain the added cation concentration at 100 mM. Oxygen consumption was assayed at 30°C for a 15-min period. The oxygen consumption observed in the presence of added cations was expressed as a percentage of that observed in the absence of Li^+, Rb^+ or Cs^+. Protein concentration was assayed in each homogenate using the biuret method as described by Gornall et al. (1949).

RbCl, CsCl and $TlNO_3$ were purchased from Ventron Alpha Products (Beverly, Mass.) and were "ultragrade". Ouabain octahydrate, yeast hexokinase, NAD, glucose and Tris-ADP were obtained from Sigma Chemical Company (St. Louis, Mo.). Nicotinamide (U.S.P.) was obtained from Merck and Company (Rahway, N.J.) and choline chloride from Eastman Organic Chemicals (Rochester, N.Y.). LiCl and other reagents used were of analytical reagent grade and were obtained from Mallinckrodt Chemical Works (St. Louis, Mo.).

Statistical analysis was performed by random design, or randomized complete block analysis of variance. The Student-Newman-Keuls test was used to determine significant differences between means. The accepted level of significance was a P-value of less than 0.05.

3. Results

3.1. Effects of Rb^+ and Cs^+

The Ca^{2+}-free Krebs—Henseleit solution used in the present study contains 3 mM KCl. Oxygen consumption of rat brain slices in such a medium, is approximately 50—60 μmols per gram tissue (wet weight) in 30 min concentration. Under these conditions, the "basal respiration", observed either in the presence or absence of 100 mM choline chloride and therefore unrelated to Na^+,K^+-ATPase activity, is approximately 40 μmoles per gram (wet) tissue (Gubitz et al., 1977). Thus, the Na^+,K^+-ATPase-related respiration of brain slices is partially activated even when KCl is not added to the incubation medium. It is plausible that such an activation of

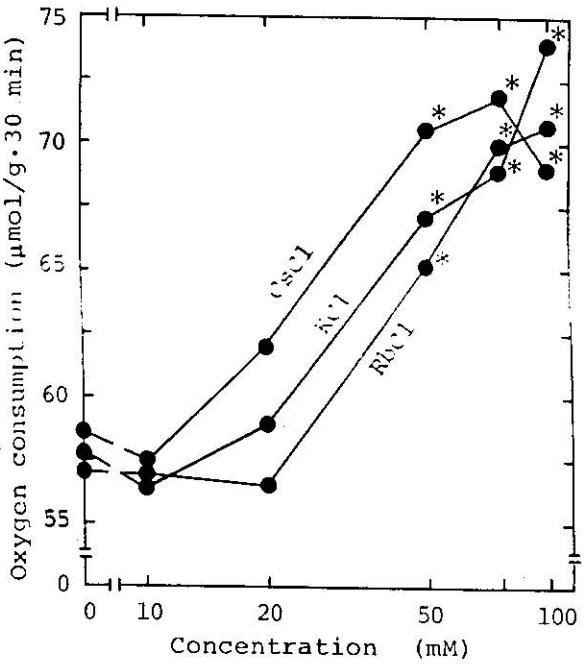

Fig. 1. Stimulation of brain slice respiration by K^+, Rb^+ or Cs^+ in a high Na^+ medium. Oxygen consumption of rat brain cortical slices was assayed at 37°C in a K^+-free medium containing 128 mM NaCl. After an equilibration period, either KCl, RbCl or CsCl was added to yield indicated final concentrations. Appropriate concentrations of choline chloride were also added so that the osmolarity was the same in each vessel following the addition. Slice respiration was then assayed for a 30-min period. Each point represents the mean of 6 experiments. * Significant stimulation by the added cation.

Na^+,K^+-ATPase in a K^+-free medium is due to the presence of intracellular K^+. When K^+ reaches the external surface of the cell membrane by passive efflux, it activates Na^+,K^+-ATPase, and is concomitantly taken up by the active cation transport mechanism, as indicated by the ability of brain slices to maintain an intracellular K^+ concentration in a K^+-free medium for a prolonged period of time. The difference between the "basal respiration" and the respiration observed in a Ca^{2+}-free medium, either in the presence or absence of KCl, therefore appears to represent the oxygen consumption as a result of the activity of Na^+,K^+-ATPase in resting cells. This oxygen uptake is approximately 15—20 μmoles per gram tissue (wet weight) in 30 min.

The further addition of KCl causes a concentration-dependent increase in the respiration rate. It has been shown previously that this K^+-induced stimulation observed in the presence of Na^+ is primarily due to an enhanced ADP production resulting from the activation of membrane $(Na^+ + K^+)$-ATPase (Whittam,1962; Whittam and Blond, 1964; Gubitz et al., 1977). Either RbCl or CsCl can substitute for KCl to stimulate brain slice respiration (fig. 1). These findings are consistent with the observation that either Rb^+ or Cs^+ can replace K^+ in the activation of membrane $(Na^+ + K^+)$-ATPase in the presence of Na^+ (Skou, 1960). Unlike the stimulation in isolated $(Na^+ + K^+)$-ATPase preparations, however, Cs^+ is apparently more potent than either K^+ or Rb^+ in enhancing slice respiration. It should also be noted that none of these cations had an effect on brain slice respiration in concentrations below 20 mM.

Ouabain, a specific inhibitor of $(Na^+ + K^+)$-ATPase, inhibits the K^+-induced enhancement of slice oxygen consumption (Whittam, 1962; Whittam and Blond, 1964). Similarly, ouabain at concentrations of 10 and 100 μM significantly inhibits the slice oxygen consumption stimulated by 100 mM RbCl (fig. 2; left panel). In addition to the inhibition of $(Na^+ + Rb^+)$-stimulated portion of the slice respiration (open bars), 100 μM ouabain also inhibits respiration in the absence of RbCl (shaded bars). Similar results were observed when 100 mM CsCl was used instead of RbCl (fig. 2; right panel). While the inhibiting effect of 1 μM ouabain on $(Cs^+ + Na^+)$-stimulated respiration was not observed under the conditions of fig. 2, it was possible to demonstate an effect of 1 μM ouabain with a different experimental design. These studies are shown in fig. 3. In these experiments, the slices were exposed to 1 μM ouabain during an initial 20-min equilibration and a subsequent 30-min control observation period prior to the addition of CsCl. The addition of CsCl in the absence of ouabain causes a significant stimulation of oxygen consumption which was observed during the entire 120-min observation period. In the presence of 1 μM ouabain, the Cs^+-induced stimulation was slightly smaller during the first 30-min period, but progressively decreases during subsequent observation periods, approaching the non-stimulated rate.

The observation that ouabain antagonizes the $(Na^+ + Rb^+)$- or $(Na^+ + Cs^+)$-stimulated respiration further supports the contention that high concentrations (100 mM) of Rb^+ or Cs^+ stimulate $(Na^+ + K^+)$-ATPase in brain slices. Moreover, Rb^+ and Cs^+ had similar effects on respiration and apparently on $(Na^+ + K^+)$-ATPase in intact cells.

The stimulation of oxygen consumption by either Rb^+ or Cs^+ in a high Na^+ medium indicates that these cations can substitute for K^+. Whether these cations are stimulatory in the absence of Na^+ was examined next. The oxygen consumption in a Na^+-free medium containing a high concentration of K^+ was lower than that observed in a high Na^+, low K^+ medium (compare figs. 1 and 4) as reported earlier (Gubitz et al., 1977). The addition of NaCl (final concentration , 100 mM) significantly stimulated respiration in this medium, whereas the addition of choline chloride had no significant effect (fig. 4). Similarly, neither RbCl nor CsCl (final concentrations, 100 mM) affected respiration in a Na^+-free medium con-

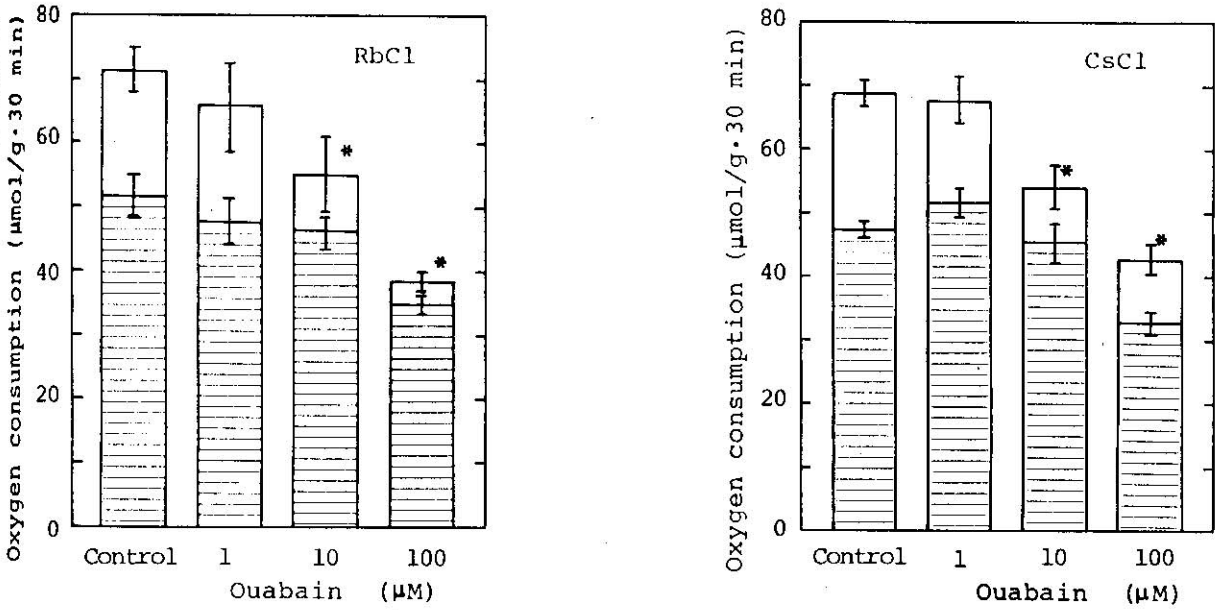

Fig. 2. Effects of Rb^+, Cs^+ and ouabain on brain slice respiration. Cortical slices were incubated at 37°C for 30 min in a medium containing 128 mM NaCl, 3 mM KCl and either 0, 1, 10 or 100 μM ouabain. RbCl (left panel), CsCl (right panel) or choline chloride was then added in final concentrations of 100 mM. Slice respiration during the first 30-min period following the addition is shown. Total bar represents the respiration in 100 mM RbCl or CsCl and shaded bar, the respiration in 100 mM choline chloride. Thus, open bar represents the $(Na^+ + Rb^+)$- of $(Na^+ + Cs^+)$-stimulated portion. Vertical lines indicate the S.E.M. of 4 experiments. * Significant inhibition by ouabain.

taining a high concentration of K^+. The addition of the same concentration of LiCl caused an inhibition of oxygen consumption under these experimental conditions. Thus, neither Rb^+, Cs^+ nor Li^+ can substitute for Na^+ in the presence of a high concentration of K^+.

3.2. *Effects of Li^+*

Previous studies (Tobin et al., 1974; Han et al., 1976) indicate that Li^+ is capable of substituting for either Na^+ or K^+ in certain of the partial reactions of isolated $(Na^+ + K^+)$-ATPase. In particular, Li^+ stimulates $(Na^+ + K^+)$-ATPase activity in the presence of Na^+ and relatively low concentrations of K^+ (Tobin et al., 1974). Thus, the effect of Li^+ on slice respiration was examined in a medium containing 150 mM NaCl and 1.5 mM KCl. Under these experimental conditions, LiCl in concentrations of 1—10 mM appears to increase oxygen consumption during the first and second 30-min observation periods (fig. 5). Even if real, these effects of Li^+, however, were transient and disappeared at later observation times. A higher concentration of LiCl (30 mM) actually inhibits respiration. This inhibitory effect of LiCl increases with time. Thus, the action of LiCl is concentration dependent; it would appear to enhance brain slice respiration in a high Na^+, low K^+ medium at low concentrations and progressively inhibits it at high concentrations. In the presence of high concentrations of Li^+, the inhibitory effect undoubtedly masks the stimulatory action of this cation.

Li^+ and Na^+ share common characteristics with respect to their effects on isolated $(Na^+ + K^+)$-ATPase (Skou, 1960; Post et al., 1972; Tobin et al., 1974; Han et al., 1976), although Li^+ is incapable of fully activating the ATPase reaction in the presence of K^+, Mg^{2+} and ATP.

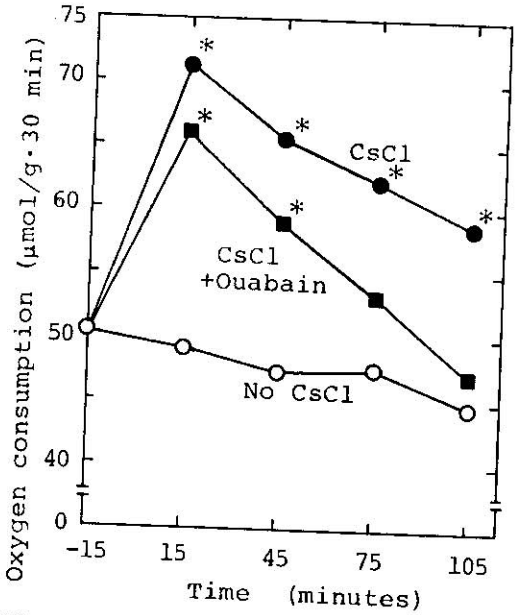

Fig. 3. Effect of ouabain on ($Na^+ + Cs^+$)-stimulated respiration of brain slices. Oxygen consumption of cortical slices was assayed at 37°C for a 30-min period in a medium containing 128 mM NaCl and 3 mM KCl with either no (circles) or 1 μM ouabain (squares). At time zero, CsCl (closed symbols) or choline chloride (open symbols) was added to the incubation mixture in a final concentration of 100 mM, and the oxygen consumption was assayed for additional four 30-min periods. Values obtained after the addition of choline chloride in the presence and absence of ouabain were pooled since this concentration of ouabain failed to affect non-stimulated respiration. The mean value of 4 experiments was plotted against the time corresponding to the midpoint of each observation period. * Significant stimulation of oxygen consumption by Cs^+.

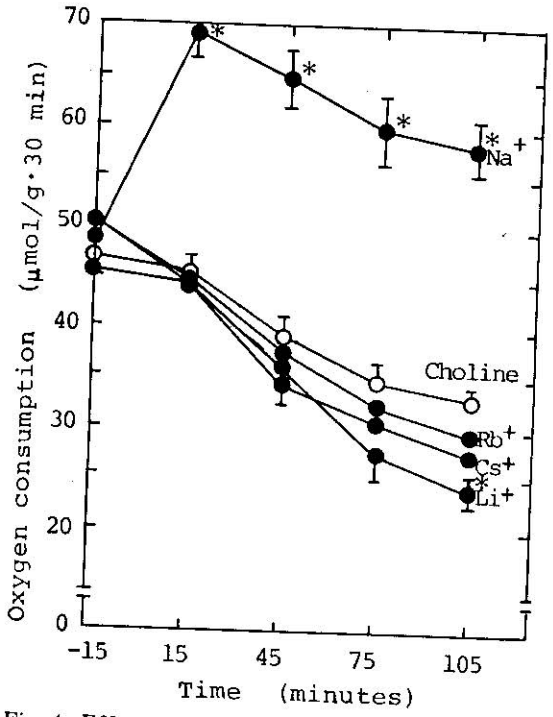

Fig. 4. Effects of Na^+, Rb^+, Cs^+ and Li^+ on brain slice respiration in a Na^+-free medium containing K^+. Oxygen consumption was assayed at 37°C for a control half-hour period in a Na^+-free medium containing 100 mM KCl. Subsequently either NaCl, choline chloride, RbCl, CsCl or LiCl was added to the incubation mixture at time zero in the final concentration of 100 mM, and the oxygen consumption was assayed for additional four 30-min periods. The mean value of 8 experiments is plotted against the time corresponding to the midpoint of each observation period. Vertical lines indicate representative S.E.M. * Significantly different from corresponding control values observed after the addition of choline chloride.

Therefore, it seemed reasonable to determine whether high concentrations of Li^+ inhibits respiration by competing with Na^+. In a Na^+-free medium containing a high concentration of K^+, the addition of Na^+ to the medium causes a concentration-dependent increase in oxygen consumption (fig. 6). The addition of Li^+ fails to stimulate respiration (values at 0 mM NaCl in fig. 6). This finding indicates that Li^+ cannot substitute for Na^+ to stimulate respiration in a high K^+ medium. The presence of 20 mM LiCl actually decreases the Na^+-stimulated respiration in a high K^+ medium. At the concentrations of Li^+ and Na^+ studied, the data did not reveal that Li^+ produced a change in the slope of the regression line, decreased the maximal rate of Na^+-stimulated respiration, or caused a parallel shift to the right in the Na^+-stimulation curve. It was thus not possible to ascertain whether the effect of Li^+ was competitive or non-competitive with respect to Na^+.

In further attempts to elucidate the mechanism by which Li^+ inhibits ($Na^+ + K^+$)-stimu-

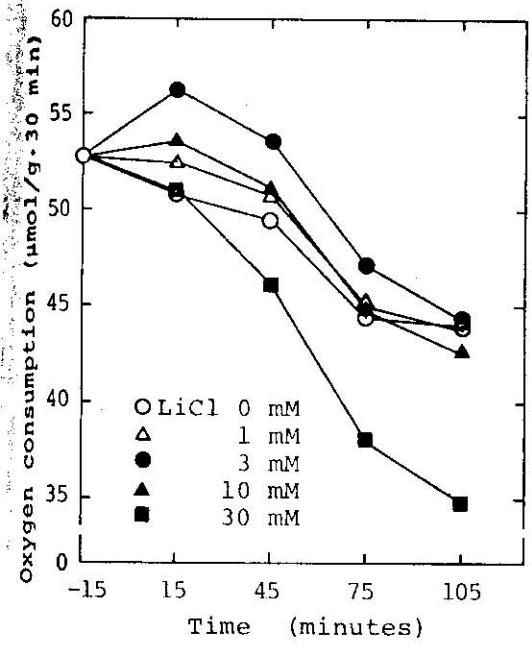

Fig. 5. Effects of Li^+ on brain slice respiration in a high Na^+, low K^+ medium. Oxygen consumption was assayed at 37°C for a control 30-min period in a medium containing 150 mM NaCl and 1.5 mM KCl. At time zero, LiCl was added to the incubation mixture in the indicated final concentrations and the oxygen comsumption was assayed for additional four 30-min periods. The mean value of 3 experiments is plotted against the time corresponding to the midpoint of each observation period.

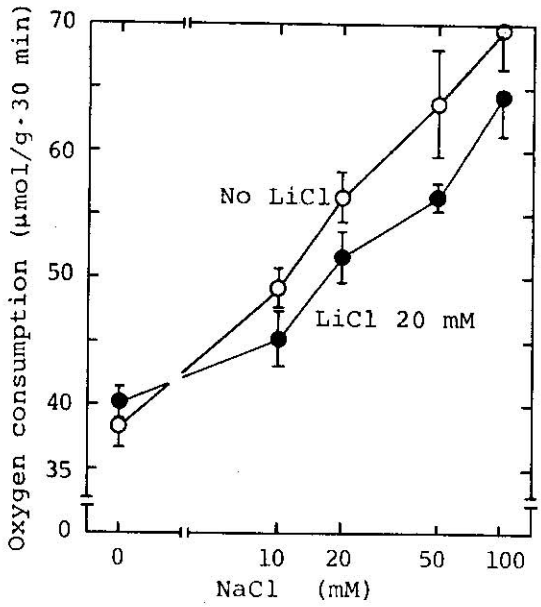

Fig. 6. Effects of Li^+ on Na^+-stimulated respiration in a high K^+-medium. Cortical slices were incubated at 37°C for 30 min in a Na^+-free medium containing 100 mM KCl and either 0 or 20 mM LiCl. NaCl was then added. Appropriate concentrations of choline chloride were also added so that the osmolarity was the same in each vessel following the addition. Slice respiration was then assayed for a 30-min period. Each point represents the mean of 4 experiments. Vertical lines indicate S.E.M.

lated respiration, the effects of Li^+ on the state 3 respiration of brain homogenates were studied (fig. 7). Under these experimental conditions, brain mitochondria are consuming oxygen at a high rate in the presence of optimal substrate, phosphate ions and phosphate acceptor (ADP) concentrations. The addition of LiCl in concentrations of 20—100 mM significantly inhibits homogenate respiration whereas lower concentrations of LiCl (10 mM or less) have no effect. In contrast to LiCl, neither RbCl nor CsCl has a significant effect on homogenate respiration in concentrations up to 100 mM. Thus, high concentrations of LiCl appear to directly inhibit oxygen consumption of brain mitochondria whereas similar concentrations of RbCl or CsCl, or low concentrations of LiCl do not have such effects.

3.3. *Effects of Tl^+*

In isolated enzyme systems, thallous ions have been shown to be an effective substitute for K^+ in the $(Na^+ + K^+)$-ATPase reaction, and an inhibitor of $(Na^+ + K^+)$-ATPase in the presence of Na^+ and K^+ (Inturrisi, 1969). For this reason, the action of Tl^+ on brain slice respiration was studied. In these studies, $TlNO_3$ was used instead of the chloride salt because of the relatively low water solubility of the latter compound. In a high $(Na^+ + K^+)$-medium, 3 mM $TlNO_3$ significantly inhibits respiration (fig. 8) while concentrations of $TlNO_3$ less than 1.0 mM have no effect.

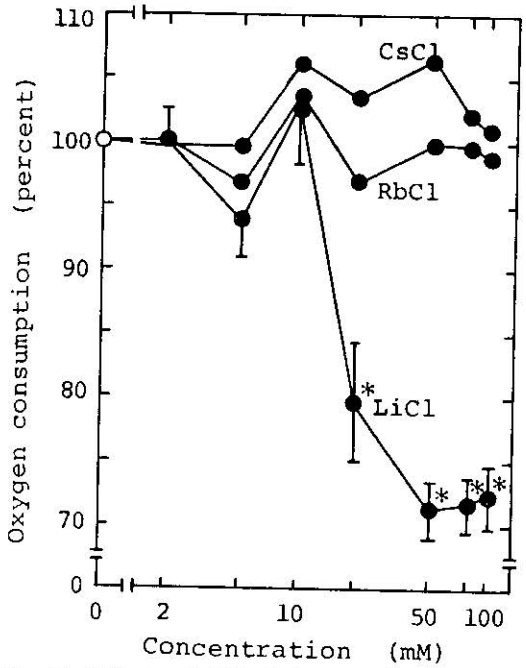

Fig. 7. Effects of Li^+, Rb^+ and Cs^+ on brain homogenate respiration. Sucrose homogenates of cortical slices were incubated at 30°C in a Na^+-free medium containing Tris-ADP, $MgCl_2$, potassium phosphate buffer (pH 7.2), glucose, EDTA, NAD, nicotinamide and hexokinase. The concentration of LiCl, RbCl or CsCl were varied from 0 to 100 mM, using choline chloride as an osmotic substitute to maintain the added cation concentration at 100 mM. Potassium concentration was approximately 25 mM. The rate of oxygen consumption in the absence of added cation (control value) was 0.34 to 0.46 μmol per gram tissue (wet weight) in 15 min. This value for each preparation was set at 100%. Each point represents the mean of 5 experiments. Vertical lines indicate representative S.E.M. * Significant inhibition compared to the control value.

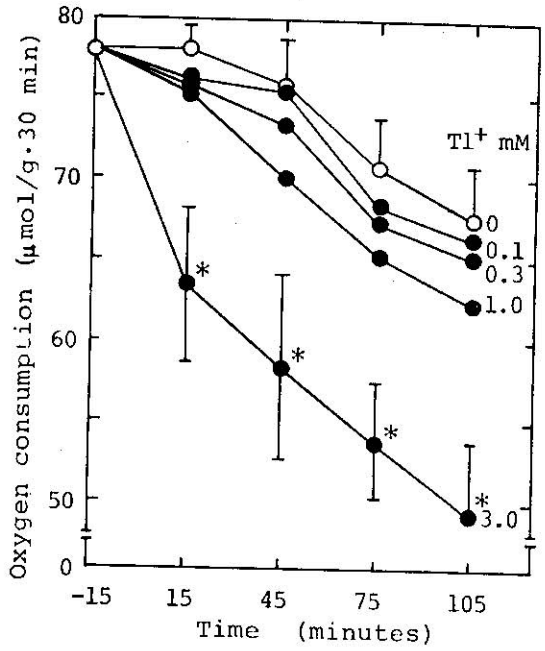

Fig. 8. Effects of Tl^+ on brain slice respiration in a high ($Na^+ + K^+$)-medium. Oxygen consumption was assayed at 37°C for a control 30-min period in a medium containing 128 mM NaCl and 100 mM KCl. Subsequently, $TlNO_3$ was added to the incubation mixture at time zero, yielding the indicated final concentrations, and the oxygen consumption was assayed for additional four 30-min periods. The mean value of 8 experiments is plotted against the time corresponding to the midpoint of each observation period. Vertical lines indicate representative S.E.M. * Significant inhibition by Tl^+.

To determine if Tl^+ is capable of substituting for K^+, the effect of Tl^+ was studied in a K^+-free medium in the presence of 128 mM NaCl. Under these conditions, however, high concentrations of Tl^+ markedly inhibits oxygen consumption (fig. 9). The inhibition caused by Tl^+ is markedly greater in the absence of K^+ (fig. 9) than in its presence (fig. 8). For example, 3.0 mM $TlNO_3$ causes a 90% inhibition in the absence of K^+ compared to a 27% inhibition in the presence of 100 mM KCl, 100 min after the addition of this cation. The inhibition in the absence of K^+ is time-dependent (fig. 9). Only during the first 30-min period, did 0.3 and 1.0 mM $TlNO_3$ produce a slight stimulation of respiration. Thus, the predominant action of Tl^+ in a high Na^+ medium containing no K^+ is the inhibition of the respiration. It is conceivable that the stimulation observed at early times with low concentrations of Tl^+ is masked during later observation periods or at high concentrations of Tl^+ by the inhibitory effect of this cation.

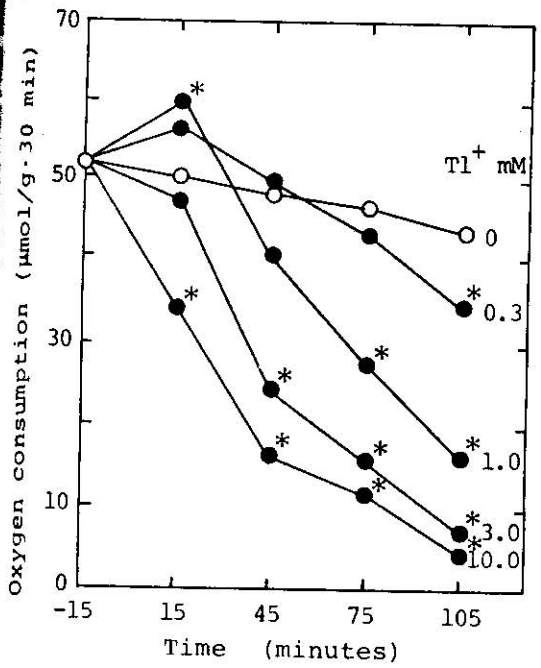

Fig. 9. Effects of Tl^+ on brain slice respiration in a K^+-free medium containing Na^+. See legend to fig. 8. A K^+-free medium containing 128 mM NaCl was used. Each point represents the mean of 4 experiments. * Significantly different from the corresponding control value.

4. Discussion

Brain slice respiration was stimulated by K^+, Rb^+ or Cs^+ in a medium containing Na^+. Cs^+ is somewhat more potent than Rb^+ or K^+, as reported in a previous study (Hertz and Schou, 1962). In isolated enzyme preparations, however, higher concentrations of Cs^+ than those of K^+ or Rb^+ are required for the activation of ATPase in the presence of 100 mM NaCl, 6 mM $MgCl_2$ and 3 mM ATP (Skou, 1960). This discrepancy might be due to differences in the environment surrounding ($Na^+ + K^+$)-ATPase and asymmetric activation of the enzyme activity by monovalent cations in intact cells. In isolated enzyme studies cation binding sites on ($Na^+ + K^+$)-ATPase in contact with the extracellular fluid (K^+-binding sites) and those facing the intracellular fluid (Na^+-binding sites) are exposed to the same medium containing a high concentration of Na^+. Thus, the affinity of K^+-binding sites for K^+, Rb^+ or Cs^+ determines the potency of these cations to stimulate ATPase activity. In contrast, the Na^+-activation of ($Na^+ + K^+$)-ATPase in intact cells takes place in the presence of the relatively low $[Na^+]_i$ which is normally antagonized by the high $[K^+]_i$. There is good evidence that internal Na^+ concentration is the principal determinant of the sodium pump activity (see Thomas, 1972). Thus, the potency of K^+, Rb^+ or Cs^+ might not be primarily determined by the affinity of K^+-binding sites for these cations in intact cells. Alternatively, K^+, Rb^+ or Cs^+ could stimulate oxygen consumption by inducing membrane depolarization. The membrane depolarization, however, is not the direct cause of the stimulation of oxygen consumption in brain slices (Gubitz et al., 1977). It has been shown that depolarization-induced increase in Na^+ influx stimulates oxygen consumption by increasing intracellular Na^+ available to sodium pump.

The concentration and time dependency of the inhibitory action of ouabain on the Rb^+- or Cs^+-stimulated respiration is similar to those actions of ouabain previously observed with K^+-stimulated respiration (Gubitz et al., 1977). In addition, only those combinations of monovalent cations that activate ($Na^+ + K^+$)-ATPase increased oxygen consumption in the present study. These data thus confirm our previous observation (Gubitz et al., 1977) that Na^+,K^+-ATPase activity can be estimated in intact cells by measuring the oxygen consumption of brain slices.

The inhibition of nonstimulated slice respiration by high concentrations of ouabain (fig. 2) is due to the accumulation of $[Na^+]_i$, which in turn directly inhibits oxidative phosphorylation (Gubitz et al., 1977). In a similar fashion, Li^+ inhibits oxygen consumption of brain homogenates, indicating that intracellular Li^+ accumulation also reduces slice respiration. Such results were observed when high concentrations of LiCl were added to a Na^+-free medium containing 100 mM KCl

(fig. 4) or to a medium containing 150 mM NaCl and 1.5 mM KCl (fig. 5). The presence of 20 mM LiCl also reduced the Na^+-stimulated respiration observed in a high K^+ medium. Attempts to evaluate the kinetics of the antagonism of Na^+-stimulation by Li^+ were unsuccessful because of the narrow range of Na^+ concentrations that could be examined. Studies shown in fig. 7 suggest that at least a part of the effect of Li^+ is due to direct inhibition of oxidative phosphorylation and hence might be non-competitive with respect to Na^+.

Low concentrations of Tl^+ stimulated brain respiration in a K^+-free medium containing 128 mM NaCl. Since the increased respiration was not observed in the presence of K^+, such an effect of Tl^+ results from the ability of this ion to substitute for K^+ in stimulating ATPase activity (Inturrisi, 1969) and hence would not occur in vivo where K^+ is present in concentrations which are not rate-limiting for the $(Na^+ + K^+)$-ATPase reaction (Schwartz et al., 1975). The stimulation was observed only with low concentrations of Tl^+ and during the earlier observation period, indicating that this stimulatory action of Tl^+ is masked by the predominantly inhibitory action of this cation at higher concentrations and during later observation periods. The primary mechanism for the Tl^+-induced inhibition is probably direct damage to mitochondria, similar to that previously reported in cultured nervous tissue (Spencer et al., 1973). In addition, Tl^+ could be reducing oxygen consumption by inhibiting $(Na^+ + K^+)$-ATPase in the presence of Na^+ and K^+. This latter effect, however, cannot account for the marked reduction in respiration observed in the absence of K^+. The inhibitory action of Tl^+ was significantly greater in the absence of K^+ than in its presence, indicating that the access or the binding of Tl^+ to its inhibitory sites is antagonized by K^+. The above results obtained with Li^+ and Tl^+, thus, indicate the limitations of the current method to assess Na^+,K^+-ATPase activity from slice respiration data. If the test agent interferes with the normal process of oxidative phosphorylation, the results should be carefully evaluated.

At high concentrations, Cs^+ and Rb^+ stimulated slice oxygen consumption. At lower concentrations, however, these cations failed to have any effect indicating that therapeutic concentrations of Rb^+ probably do not affect the $(Na^+ + K^+)$-ATPase activity. Thus, the present study does not provide supportive evidence for the contention that the antidepressive action of Rb^+ is due to its inhibition of brain $(Na^+ + K^+)$-ATPase activity. In contrast, low concentrations of Li^+ apparently stimulated the oxygen consumption under certain conditions, consistent with the action of this cation on isolated $(Na^+ + K^+)$-ATPase activity. The effect of Li^+ to stimulate isolated $(Na^+ + K^+)$-ATPase (Spring, 1976; Han et al., 1976) is presumably due to uncoupling of the transport of monovalent cations from the hydrolysis of ATP (Willis and Fang, 1970; Post et al., 1972; Tobin et al., 1974). Brain or kidney slices leached and incubated in a Na^+-free medium containing either 125 mM LiCl (Hertz and Schou, 1962) or 140 mM LiCl (Willis and Fang, 1970) have been shown to have a transient high respiration rate. The therapeutic concentration of Li^+ in plasma, however, is in the range of 0.6 to 1.5 mEq/liter with toxic effects seen at higher concentrations (Gershon, 1970). Thus, the concentration of Li^+ which caused a stimulation of brain slice respiration (3 mM) would normally be toxic. In addition, the stimulation was only transient whereas the therapeutic effect of Li^+ is observed only after chronic administration of the agent (Gershon, 1970). Moreover, the effect of Li^+ on membrane function could be an inhibition of sodium pump activity, similar to that of Rb^+, despite the stimulation of ATP utilization and respiration (Willis and Fang, 1970; Ku et al., 1975, 1978).

In conclusion $(Na^+ + K^+)$-ATPase activity per se does not appear to be involved in the central nervous system effects of Li^+ or Rb^+ despite the fact that this enzyme system is capable of sharply discriminating between these two cations.

Acknowledgements

This work was supported by a National Science Foundation grant, BMS 74-18512 A01, and U.S. Public Health Service grants, HL 16052 from the National Heart, Lung and Blood Institute and DA 01881 from the National Institute on Drug Abuse.

References

Dick, D.T.A., G.J. Naylor, E.G. Dick and J.P. Moody, 1974, Erythrocyte sodium-plus-potassium ion-activated adenosine triphosphatase in depressive patients, Biochem. Soc. Trans. 2, 505.

Fieve, R.H., H. Meltzer, D.L. Dunner, M. Levitt, J. Mendlewicz and A. Thomas, 1973, Rubidium: Biochemical, behavioral and metabolic studies in humans, Amer. J. Psychiat. 130, 55.

Frazer, A., J. Mendels, D. Brunswick and T.A. Ramsey, 1978, Transfer of sodium ions across the erythrocyte membrane in manic-depressive illness: Treatment with lithium carbonate, Life Sci. 22, 157.

Gershon, W., 1970, Lithium in mania, Clin. Pharmacol. Therap. 11, 168.

Glen, A.I.M. and H.W. Reading, 1973, Regulatory action of lithium in manic-depressive illness, Lancet 2, 1239.

Glen, A.I.M., G.C. Ongley and K. Robinson, 1968, Diminished membrane transport in manic-depressive psychosis and recurrent depression, Lancet 2, 241.

Gornall, A.G., C.J. Bardawill and M.M. David, 1949, Determination of serum protein by means of the biuret reaction, J. Biol. Chem. 177, 751.

Gubitz, R.H., T. Akera and T.M. Brody, 1977, Control of brain slice respiration by $(Na^+ + K^+)$-activated adenosine triphosphatase and the effects of enzyme inhibitors, Biochim. Biophys. Acta 459, 263.

Gutman, Y., S. Hochman and H. Wald, 1973, The differential effect of Li^+ on microsomal ATPase in cortex, medulla and papilla of the rat kidney, Biochim. Biophys. Acta 298, 284.

Gutman, Y., S. Hochman and D. Strachman, 1973, Effect of lithium treatment on microsomal ATPase activity in several tissues, Intern. J. Biochem. 4, 315.

Han, C.S., T. Tobin, T. Akera and T.M. Brody, 1976, Effects of alkali metal cations on phospho-enzyme levels and (^3H)-ouabain binding to $(Na^+ + K^+)$-ATPase, Biochim. Biophys. Acta 429, 993.

Hertz, L. and M. Schou, 1962, Univalent cations and the respiration of brain-cortex slices, Biochem. J. 85, 93.

Hesketh, J.E., 1976, Changes in membrane adenosine triphosphatases on administration of lithium salts in vivo, Biochem. Soc. Trans. 4, 328.

Hesketh, J.E., N. Kinloch and H.W. Reading, 1977, The effects of lithium on ATPase activity in subcellular fractions from rat brain, J. Neurochem. 29, 883.

Hokin-Neaverson, M., D.A. Spiegel and W.C. Lewis, 1974, Deficiency of erythrocyte sodium pump activity in bipolar manic-depressive psychosis, Life Sci. 15, 1739.

Inturrisi, C.E., 1969, Thallium induced dephosphorylation of phosphorylated intermediate of the (sodium + thallium-activated) ATPase, Biochim. Biophys. Acta 179, 630.

King, L.J., J.L. Carl, E.G. Archer and M. Castelianet, 1969, Effects of lithium on brain energy reserves and cations in vivo, J. Pharmacol. Expt. Therap. 168, 163.

Ku, D.D., T. Akera, T. Tobin and T.M. Brody, 1975, Effects of monovalent cations on cardiac Na^+,K^+-ATPase activity an on contractile force, Naunyn-Schmiedeb. Arch. Pharmacol. 290, 113.

Ku, D.D., T. Akera, M.K. Olgaard and T.M. Brody, 1978, Effects of lithium and thallous ions on sodium pump activity in the guinea-pig heart and their relationship to the positive inotropic action, Naunyn-Schmiedeb. Arch. Pharmacol. 304, 167.

McNulty, J., D.J. O'Donovan and B.E. Leonard, 1975, The effects of d-amphetamine, chlorpromazine, amitriptyline and lithium chloride on adenosine 5′-triphosphatases in ten regions of the rat brain, Biochem. Soc. Trans. 3, 1229.

Meltzer, H.L., R.M. Taylor, S.R. Platman and R.R. Fieve, 1969, Rubidium: A potential modifier of affect and behaviour, Nature 223, 321.

Mendels, J. and A. Frazer, 1974, Alterations in cell membrane activity in depression, Amer. J. Psychiat. 131, 1240.

Mendels, J., S. Stern and A. Frazer, 1976, Biochemistry of depression, Dis. Nerv. Syst. 37, 3.

Naylor, G.J., H.B. McNamee and J.P. Moody, 1970, The plasma control of erythrocyte sodium and potassium metabolism in depressive illness, J. Psychosom. Res. 14, 179.

Naylor, G.J., D.A.T. Dick, E.G. Dick, D. Lepoidevin, and S.F. Whyte, 1973, Erythrocyte membrane cation carrier in depressive illness, Psychol. Med. 3, 502.

Naylor, G.J., D.A.T. Dick, E.G. Dick, E.P. Worrall, M. Peet, P. Dick and L.J. Boardman, 1976, Erythrocyte membrane cation carrier in mania, Psychol. Med. 6, 659.

Pappius, H.M. and K.A.C. Elliot, 1956, Factors affecting the potassium content of incubated brain slices, Can. J. Biochem. Physiol. 34, 1053.

Ploeger, E.J., 1974a, The effects of lithium on excit-

able cell membranes. On the mechanism of inhibition of the sodium pump of non-myelinated nerve fibers of the rat, European J. Pharmacol. 25, 316.

Ploeger, E.J., 1974b, The effects of lithium on excitable cell membrane. The influence on the ATPase of homogenates of the non-myelinated nerve fibers of the rat, Arch. Intern. Pharmacodyn. Therap. 210, 374.

Post, R.L., C. Hegyvary and S. Kume, 1972, Activation by adenosine triphosphate in the phosphorylation kinetics of Na and K ion transport adenosine triphosphatase, J. Biol. Chem. 247, 6530.

Potter, V.R., 1972, The homogenate technique, in: Manometric and Biochemical Techniques, eds. W.W. Umbreit, R.H. Burris and J.F. Stauffer (Burgess Publishing Company, Minnesota) p. 177.

Schwartz, A., G.E. Lindenmayer and J.C. Allen, 1975, The sodium-potassium adenosine triphosphatase: Pharmacological, physiological and biochemical aspects, Pharmacol. Rev. 27, 3.

Skou, J.C., 1960, Further investigations on a Mg^{++} + Na^{+}-activated adenosine triphosphatase, possibly related to the active, linked transport of Na^{+} and K^{+} across the nerve membrane, Biochim. Biophys. Acta 42, 6.

Spencer, P.S., E.R. Peterson, R.A. Madrid and C.S. Raine, 1973, Effects of thallium salts on neuronal mitochondria in organotype cord-ganglia-muscle combination cultures, J. Cell Biol. 58, 79.

Spring, G.K., 1976. The current role of lithium in the treatment of affective disorders, Psychosomatics 17, 151.

Tobin, T., T. Akera, C.S. Han and T.M. Brody, 1974, Lithium and rubidium interactions with sodium- and potassium-dependent adenosine triphosphatase: a molecular basis for the pharmacological actions of these ions, Mol. Pharmacol. 10, 501.

Thomas, R.C., 1972, Electrogenic sodium pump in nerve and muscle cells, Physiol. Rev. 52, 563.

Umbreit, W.W., 1972, Constant volume manometry – the "Warburg", in: Manometric and Biochemical Techniques, eds. W.W. Umbreit, R.H. Burris and J.F. Stauffer (Burgess Publishing Company, Minnesota) p. 1.

Whittam, R., 1962, The dependence of the respiration of brain cortex on active cation transport, Biochem. J. 82, 205.

Whittam, R. and M.E. Ager, 1964, Vectoral aspects of adenosine-triphosphatase activity in erythrocyte membranes, Biochem. J. 93, 337.

Whittam, R. and D.M. Blond, 1964, Respiratory control by an adenosine triphosphatase involved in active transport in brain cortex, Biochem. J. 92, 147.

Willis, J.S. and L.S.T. Fang, 1970, Li^{+} stimulation of ouabain-sensitive respiration and (Na^{+}-K^{+})-ATPase of kidney cortex of ground squirrels, Biochim. Biophys. Acta 219, 486.