Biochemistry

 Volume 29, Number 3 January 23, 1990

Articles

NMR Characteristics of Intracellular K in the Rat Salivary Gland: A ^{39}K NMR Study Using Double-Quantum Filtering

Yoshiteru Seo,*,‡ Masataka Murakami,‡ Eiji Suzuki,‡ Satoru Kuki,‡ Kuniaki Nagayama,§ and Hiroshi Watari‡

Department of Molecular Physiology, National Institute for Physiological Sciences, Myodaiji, Okazaki 444, Japan, and Biometrology Laboratory, JEOL Ltd., Akishima, Tokyo 196, Japan

Received January 19, 1989; Revised Manuscript Received September 7, 1989

ABSTRACT: Intracellular K of the perfused rat mandibular salivary gland was measured by ^{39}K NMR spectroscopy at 8.45 T. Multiple-quantum NMR arising from multiple-exponential decay was used to eliminate the resonance due to extracellular K in the perfused gland at 25 °C. The resonance due to intracellular K consisted of two Lorentzian signals stemming from the $|^1/_2><-^1/_2|$ coherence (sharp resonance) and the $|-^1/_2><-^3/_2|$, $|^3/_2><^1/_2|$ coherences (broad resonance). The transverse relaxation time (T_2) corresponding to the $|^1/_2><-^1/_2|$ coherence was ca. 2.5 ms, and that corresponding to the $|-^1/_2><-^3/_2|$, $|^3/_2><^1/_2|$ coherences was ca. 0.4 ms. The relaxation time of the double-quantum coherence of rank 3 (originating from product operators like $I_x^2I_z$) was determined to be ca. 0.2 ms. These results suggest the possibility of the presence of a single homogeneous population of intracellular K with a correlation time of ca. 2.5×10^{-8} s and a quadrupolar coupling constant of ca. 1.4 MHz.

In a previous paper (Seo et al., 1987b), we reported the application of ^{39}K nuclear magnetic resonance (NMR) spectroscopy to the perfused rat mandibular salivary gland. In that study, we found a significant difference between the longitudinal relaxation (T_1) times of extracellular and intracellular K. The T_1 value of the intracellular K was ca. 3 ms, which could be explained by an effective viscosity of the intracellular fluid of 50 cP. This value is much higher than previous estimates of the viscosity of intracellular fluid (1–5 cP) (Heilbrunn, 1938) and suggests the presence of some additional interaction between K and its intracellular environment. Consequently, we tried to precisely determine the longitudinal (T_1) and transverse (T_2) relaxations for the intracellular K and tried to detect two T_1 and two T_2 values. Since ^{39}K has a spin of $^3/_2$, the quadrupole relaxation process might be responsible for both the fast T_1 and T_2 relaxations and the biexponential relaxation. High molecular weight solutes such as proteins would be expected to cause the observed enhancement in the relaxation rate of ^{39}K coherence. Recently, the method of double-quantum filtered NMR spectroscopy has been reported (Bax et al., 1980; Piantini et al., 1982; Shaka & Freeman, 1983; Jaccard et al., 1986; Rooney et al., 1988). Pekar et al. (1986, 1987) have reported applications of the T_2 sequence by double-quantum filtering to ^{23}Na in albumin solutions and to the intracellular Na of erythrocytes. This method is selective for the double-quantum coherence of rank 3 arising from the $|^1/_2><-^3/_2|$, $|^3/_2><-^1/_2|$ coherences. We have detected also a resonance corresponding to the double-quantum transition of ^{39}K in the perfused rat mandibular gland and have obtained relaxation parameters and have estimated the correlation time of the intracellular K.

Experimental Procedures

Preparation of the Isolated Salivary Gland. The mandibular salivary glands of the rat (Wistar-Hamamatsu, weighing 250–350 g) were isolated from the rats anesthetized with sodium pentobarbital (50 mg/kg of body weight, intraperitoneal injection). The glands (weighing 0.2 g) were placed in an NMR tube (10-mm diameter) and perfused arterially with a modified Krebs solution at a rate of 2 mL/min with a peristaltic pump (Cole-Palmer) (Murakami et al., 1987). The composition of the modified Krebs solution was 146 mM Na, 4.3 mM K, 1 mM Ca, 1 mM Mg, 148.3 mM Cl, 5 mM glucose, and 10 mM Hepes buffer (pH 7.4), and it was saturated with 100% O_2. The composition of the perfusate containing dysprosium triethylenetetraamine-*N*,*N*′,*N*″,*N*‴,-

‡National Institute for Physiological Sciences.
§JEOL Ltd.

0006-2960/90/0429-599$02.50/0

N'''-hexaacetic acid [Dy(TTHA)] was 10 mM Dy(TTHA), 146 mM Na, 4.3 mM K, 1 mM Ca, 1 mM Mg, 118.4 mM Cl, 5 mM glucose, and 10 mM Hepes buffer (pH 7.4). The space outside the gland was superfused with a 300 mM sucrose solution.

Measurement of ^{39}K NMR. ^{39}K NMR spectra were collected on a WM-360wb spectrometer (Bruker, 8.45 T) with a ^{39}K-selective probe tuned to 16.8 MHz. The T_2 sequence of the double-quantum filter, D–90°–τ/2–180°–τ/2–90°–δ–90°–acquire, was used with a 32-step phase cycle. τ was the creation time (0.1–8 ms), δ was the double-quantum evolution time (0.01–10 ms), and D was the relaxation delay (0.4–0.15 s). In every experiment, the 180° pulse width was measured precisely (75–78 μs). The spectral width was 10 or 42 kHz, the receiver dead time after the last 90° pulse was 25 or 6 μs, and 2048 data points were used. Two to fourteen experiments were performed according to the same protocol, and the free induction decays were added with an additive data transfer routine (DISNMR88, Bruker). The data were stored on a magnetic tape (T-45, CDC) and then transferred to a MS-DOS format by a microcomputer (HP-9816, Hewlett-Packard), and the data were analyzed with a microcomputer (PC-9801 RA, NEC) using 1-2-3 (Lotus) software routines.

The longitudinal (T_1) and transverse (T_2) relaxation times of the intracellular K in the perfused gland were also measured by conventional methods of the inversion recovery (D1–180°–VD–90°–acquire) and spin-echo (D1–90°–VD/2–180°–VD/2–acquire) pulse sequences, respectively, where D1 was the relaxation delay (0.4 s) and VD was the variable delay (1–45 ms and 0.4 s). The spectral width was 10 kHz, the receiver dead time was 25 μs, and 2048 data points were used. Four to seven experiments were performed with the same protocol, and the data were added. The Fourier transform was then applied with a line-broadening factor of 15 Hz after the data block was extended to 4096 points. The intracellular K resonance was separated from the extracellular resonance by the line shape analysis method reported previously (Seo et al., 1987a,b).

Results

Before applying the double-quantum filter to the salivary gland, we carried out preliminary experiments with simple aqueous solutions. The detectable magnetization $M_x(\tau,\delta,t)$ under the on-resonance condition is (Pekar & Leigh, 1986; Rooney et al., 1988)

$$M_x(\tau,\delta,t) = 0.75(e^{-s_2\tau} - e^{-s_1\tau})e^{-s_{dq}\delta}(e^{-s_2 t} - e^{-s_1 t}) \quad (1)$$

where τ is the creation time, δ is the evolution time, t is the time following the detection pulse (t includes the receiver dead time after the last 90° pulse), s_{dq} is the double-quantum relaxation rate constant arising from $|^1/_2><^{-3}/_2|$, $|^3/_2><^{-1}/_2|$ rank 3 coherences, and s_1 and s_2 are the transverse relaxation rate constants ($1/T_2$) of the $|^{-1}/_2><^{-3}/_2|$, $|^3/_2><^1/_2|$ coherences and the $|^1/_2><^{-1}/_2|$ coherence, respectively. In the condition of extreme narrowing ($\omega\tau_c \ll 1$, where ω is the Larmor frequency and τ_c is the correlation time), the relaxation rate constants of s_1 and s_2 have the same value. The double-quantum filter will therefore not evoke a double-quantum coherence. Preliminary observation on the Krebs solution showed no significant signal from the double-quantum relaxation and showed an artifact of dispersion-like line shape at 0.3% of the intensity obtained with the one pulse sequence.

When $\omega\tau_c$ is around or larger than 1 (the slow-motion condition), the quadrupole relaxation causes a significant difference in the transverse relaxation rate constants, s_1 and s_2. Hence, a double-quantum coherence is evoked by the double-quantum filter (Jaccard et al., 1986; Rooney et al., 1988). Figure 1a shows the ^{39}K double-quantum filtered spectrum obtained from a bovine albumin solution (Sigma A6003, 0.5 g in 0.5 mL of 2 M KCl solution, ca. 57% v/v). The spectrum consisted of two Lorentzian peaks in antiphase. The line widths at the half-heights of the peaks ($\nu_{1/2}$) were ca. 90 and 30 Hz, and the spectrum had a vanishing integral (Figure 1b). Thus, the resonance consisted of two resonances in antiphase with the same integral.

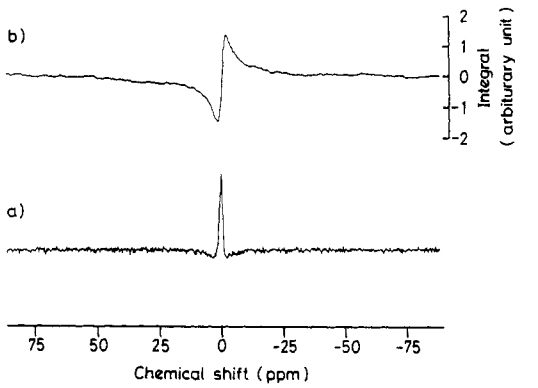

FIGURE 1: (a) A double-quantum filtered spectrum of an albumin solution (0.5 g of albumin in 1 mL of 2 M KCl solution, ca. 57% v/v) obtained with an evolution time (δ) of 10 μs, a creation time (τ) over the range from 0.5 to 36 ms, and an effective dead time after the sampling pulse of 25 μs. Since spectra obtained with τ over the range from 0.5 to 36 ms showed the same line shape, an accumulative spectrum is shown. The total number of scans was 40960. The Fourier transform was applied with a line broadening of 5 Hz. The spectrum consists of two Lorentzian lines in antiphase corresponding to the $|^{-1}/_2><^{-3}/_2|$, $|^3/_2><^1/_2|$ coherences and the $|^1/_2><^{-1}/_2|$ coherence. (b) The net integral of the spectrum. The spectrum had a vanishing integral.

The form of eq 1 suggests two methods for determining the relaxation rate constants, s_1 and s_2. Figure 2b shows the change in signal intensity with τ over the range from 0.25 to 100 ms for an albumin solution, and the dotted line shows the result of fitting the function $k(e^{-s_2\tau} - e^{-s_1\tau})$ to the data. Values for s_1 and s_2 of 220 and 78 s^{-1} (correlation coefficient 0.98, n = 27) were obtained. The relative intensity (k) was approximately 10% of that obtained with the one-pulse sequence.

The free induction decay contains the same information on the relaxation rates. Figure 2a shows the signal intensity of the free induction decay with t values ranging from 0.025 to 60 ms, and the bold line shows the result of fitting $k(e^{-s_2 t} - e^{-s_1 t})$. Values for s_1 and s_2 of 290 and 89 s^{-1} (correlation coefficient 0.93, n = 500) were obtained. These results were in good agreement within the accuracy of the measurements. The relaxation rate constant of rank 3 double-quantum coherence (s_{dq}) was also obtained. Figure 3 shows the changes in peak height with δ over the range from 0.01 to 10 ms. The relaxation time course follows a single exponential, and s_{dq} was 284 ± 13 s^{-1} (regression coefficient and standard error, n = 20).

The double-quantum filtered spectrum of the perfused rat mandibular gland at 25 °C is shown in Figure 4a. Because of the low sensitivity of the double-quantum filter and of the ^{39}K nucleus, 13.7 h of accumulation was required to obtain an adequate signal-to-noise ratio. When the glands were exposed to hypoxic conditions, they did not maintain their content of intracellular K, and the concentration might decrease to that of extracellular K (4.3 mM). No resonance was detected in the spectrum of the gland perfused hypoxically (Figure 4b). To confirm the origin of the resonance observed by the double-quantum filter, we used a chemical shift reagent, Dy(TTHA) (Chu et al., 1984). Figure 5a shows a spectrum

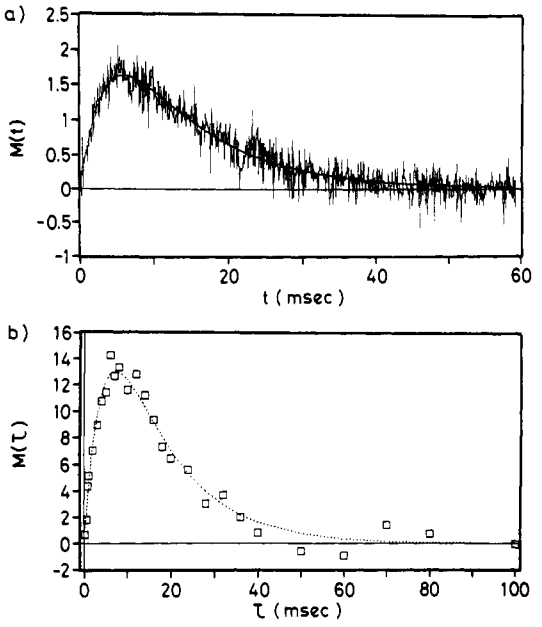

FIGURE 2: Measurements of the relaxation parameters of an albumin solution (0.5 g in 0.5 mL of 2 M KCl) at 25 °C. (a) The free induction decay observed with an evolution time (δ) of 10 μs. The signal intensity of the free induction decay [$M(t)$] is shown from 0.025 to 60 ms following the detection pulse. The bold line shows the result of fitting $k(e^{-s_2 t} - e^{-s_1 t})$ to the data over the 0.025–40-ms period. Since free induction decays obtained with τ over the range from 0.5 to 36 ms had the same shape, an accumulation free induction decay is used for the fitting. Values for s_1 and s_2 of 290 and 89 s^{-1} (correlation coefficient 0.93, n = 500) were obtained. (b) Measurement of the transverse relaxation of the $|^{-1}/_2><^{-3}/_2|$, $|^{3}/_2><^{1}/_2|$ coherences (s_1) and of the $|^{1}/_2><^{-1}/_2|$ coherence (s_2). Signal peak heights [$M(\tau)$] are measured for creation times (τ) over the range from 0.25 to 100 ms (□) with δ of 10 μs. The dotted line shows the result of fitting the function $k(e^{-s_2 \tau} - e^{-s_1 \tau})$. Values for s_1 and s_2 of 220 and 78 s^{-1} (correlation coefficient 0.98, n = 27) were obtained.

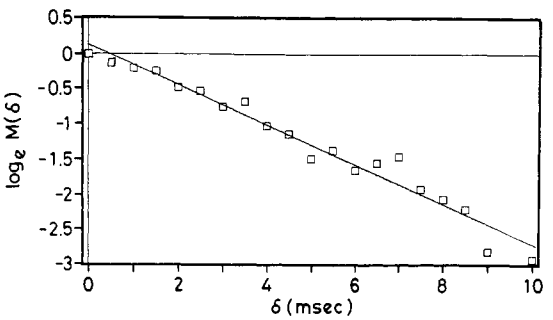

FIGURE 3: Measurements of the double-quantum relaxation of the $|^{1}/_2><^{-3}/_2|$, $|^{3}/_2><^{-1}/_2|$ coherences of an albumin solution (0.5 g in 0.5 mL of 2 M KCl) at 25 °C. Signal peak heights [$M(\delta)$] were obtained with the evolution time (δ) over the range from 0.01 to 10 ms and with a creation time (τ) of 6 ms. The relaxation rate (s_{dq}) was 284 ± 13 s^{-1} (regression coefficient and standard error, n = 20).

obtained with the one-pulse sequence. As reported previously (Seo et al., 1987b), the extracellular K resonance was shifted approximately 5.5 ppm from the intracellular K resonance (0.0 ppm) by the administration of 10 mM Dy(TTHA). Figure 5b shows a double-quantum filtered spectrum of the gland perfused with Dy(TTHA). The resonance remained its original position, corresponding to the intracellular K. No resonance corresponding to the extracellular K was observed. The possibility of contamination from extracellular K can therefore be excluded.

Figure 6a shows the free induction decay from the perfused salivary gland. Transverse relaxation rate constants of s_1 (1860, 2360, and 4060 s^{-1}) and s_2 (540, 350, and 300 s^{-1}) were obtained from three experiments. The relative intensity was

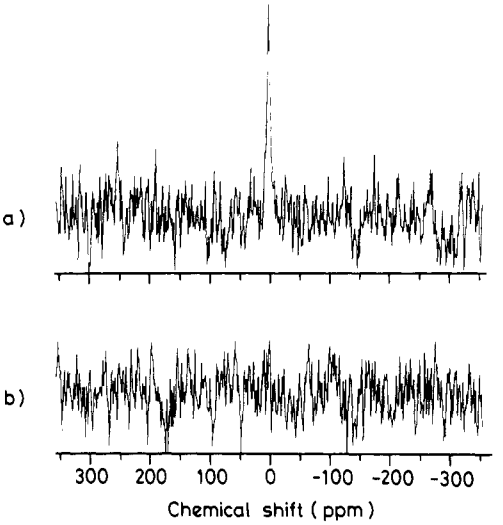

FIGURE 4: Double-quantum filtered spectra of the perfused rat mandibular gland at 25 °C under (a) aerobic and (b) hypoxic conditions. Two experiments in two glands with δ of 10 μs and τ of 0.5 ms were done, and the number of scans was 163 840 (6.85 h) in each gland. (a) Because of a long effective receiver dead time after the last 90° pulse (102 μs) and a low signal-to-noise ratio, only the sharp resonance corresponding to the $|^{1}/_2><^{-1}/_2|$ coherence is noticeable. (b) When the glands were exposed to the hypoxic condition (20% O_2/80% N_2), the gland did not maintain the level of intracellular K, and the concentration decreased. No resonance was detected.

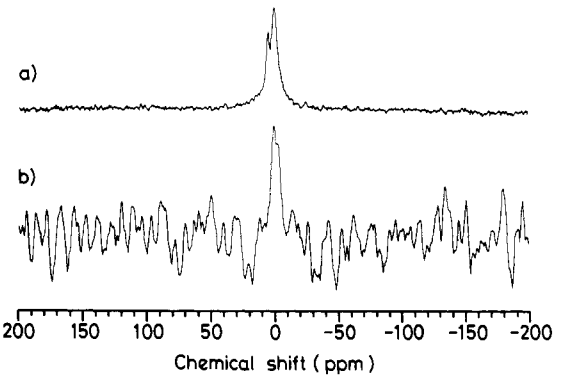

FIGURE 5: ^{39}K NMR spectra of the rat salivary gland perfused with 10 mM Dy(TTHA) at 25 °C. By use of three glands, single-pulse spectra were accumulated for 0.5 h, and then double-quantum filtered spectra were accumulated for 4.0 h, and this pair of measurements was repeated three to four times in each gland. (a) A spectrum obtained by the one-pulse sequence (D–90°–acquire) with a pulse repetition time (D) of 0.25 s and a total number of scans of 106 496. The intracellular K resonance (0.0 ppm) and the extracellular K resonance (5.5 ppm) were separated by the shift reagent. During the Dy(TTHA) perfusion, the content of the intracellular K decreased progressively and decreased by 35% at 13.5 h compared with the initial level. (b) Double-quantum filtered spectrum obtained with δ of 10 μs, τ of 0.5 ms, and a total number of scans of 729 088. Only the resonance corresponding to the intracellular K (0.0 ppm) was observed.

about 12% of the full intensity of the intracellular K resonance obtained with the one-pulse sequence. Results of an experiment with τ varied over the range from 0.05 to 8 ms were shown to be in good agreement with the free induction experiment (Figure 6a). The double-quantum coherence relaxation rate constant (s_{dq}), determined from the change in peak height with δ over the range from 0.01 to 0.4 ms (Figure 6b), had a value of 5170 ± 430 s^{-1} (regression coefficient and standard error, n = 6).

The longitudinal relaxation of the intracellular K of the perfused glands was measured by the inversion–recovery pulse sequence. The intracellular K had two T_1 components. The shorter T_1 of the intracellular K was 2.3 ms (95% confidence

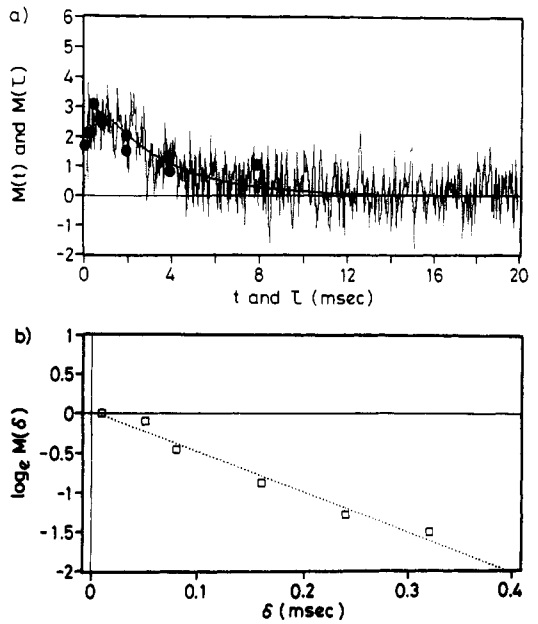

FIGURE 6: (a) The accumulated free induction decays of the perfused salivary glands obtained with the double-quantum filter. The creation time was 0.5 ms, the evolution time (δ) was 10 μs, the sampling interval was 24 μs, and the relaxation delay was 0.15 s. Nine glands were used with 6–12 h of accumulation for each gland. The solid line shows the result of fitting $k(e^{-s_2 t} - e^{-s_1 t})$ to the data over the range from 0.1 to 12 ms. The transverse relaxation rate constants, s_1 (4060 s^{-1}) and s_2 (300 s^{-1}), were obtained (correlation coefficient 0.54, n = 487). The results of experiments with τ values varied over the range from 0.05 to 8 ms (●) with δ of 10 μs using five glands are also shown. (b) The double-quantum relaxation of the $|^1/_2><^{-3}/_2|$, $|^3/_2><^{-1}/_2|$ coherences. Changes in peak height [$M(\delta)$] with evolution time (δ) over the range from 0.01 to 0.32 ms were obtained from 14 glands with a total accumulation of 598 016 transients over 41.5 h for each value of δ. The creation time was 0.5 ms, and the relaxation delay was 0.15 s. The relaxation rate (s_{dq}) was 5170 ± 430 s^{-1} (regression coefficient and standard error, n = 6).

limits were 2 and 2.8 ms, n = 5), and the longer T_1 was ca. 10 ms. The T_1 value of extracellular K was 38 ms (95% confidence limits were 36 and 40 ms, n = 8).

The transverse relaxation of the intracellular K of the perfused gland was also measured with the spin-echo pulse sequence. The T_2 values of the intra- and extracellular K were 2 ms (95% confidence limits were 1.9 and 2.3 ms, n = 6) and 16 ms (10 and 38 ms, n = 6), respectively. The shorter T_2 component corresponding to the relaxation of $|^{-1}/_2><^{-3}/_2|$, $|^3/_2><^1/_2|$ coherences could not detected because the variable delays used for the measurement were too long to detect relaxation times shorter than 1 ms.

Discussion

This is the first study in which the parameters of quadrupolar relaxation of ^{39}K have been determined in a living organ. Measurement of the three types of relaxation processes corresponding to the $|^1/_2><^{-1}/_2|$ coherence, the $|^{-1}/_2><^{-3}/_2|$, $|^3/_2><^1/_2|$ coherences, and the double-quantum coherence of rank 3 arising from the $^1/_2$ to $^{-3}/_2$, $^3/_2$ to $^{-1}/_2$ transitions is prerequisite, if the presence of quadrupolar relaxation is to be confirmed. Conventional methods have not proved so successful in detecting quadrupolar relaxation. In a previous study (Seo et al., 1987b), we failed to detect the longer T_1 component. Also, because of the low sensitivity of the ^{39}K nucleus, we could not detect the broad resonance corresponding to the $|^{-1}/_2><^{-3}/_2|$, $|^3/_2><^1/_2|$ coherences. In this study, we detected two T_1 relaxations by using the inversion–recovery pulse sequence, but we could not detect the shorter T_2 relaxation using the spin-echo pulse sequence.

Recently, Rooney et al. (1988) presented four possible types of spectra for understanding the isolated quadrupolar nuclei with a spin of $^3/_2$ in biological tissue: (a) single crystal-like, (b) inhomogeneous powder pattern, (c) homogeneous biexponential, and (d) extreme narrowed spectra. The magnitude of $\bar{\omega}_Q$ (the time-averaged values of the quadrupolar coupling constant in frequency units) is important in discriminating the inhomogeneous powder pattern and the homogeneous biexponential spectra. When $\tau_c \gg \bar{\omega}_Q^{-1}$ and $\tau_c \gg \omega^{-1}$, the resonance splits into three resonances: a central peak corresponding to the $|^1/_2><^{-1}/_2|$ transition and two inhomogeneous satellite resonances corresponding to the $|^{-1}/_2><^{-3}/_2|$ coherence and the $|^3/_2><^1/_2|$ coherence (the inhomogeneous powder pattern). When $\bar{\omega}_Q^{-1} \gg \tau_c \gg \omega^{-1}$, the resonance is a superposition of a narrow resonance corresponding to the $|^1/_2><^{-1}/_2|$ transition and a broad resonance corresponding to the $|^{-1}/_2><^{-3}/_2|$ coherence and the $|^3/_2><^1/_2|$ coherence at the same frequency (the homogeneous biexponential spectrum) (Rooney et al., 1988). Na ion in glycerol exhibits a homogeneous biexponential relaxation from a single population (Lerner & Torchia, 1986). When we assumed a single homogeneous population of K in the albumin solutions, the correlation time of K and the quadrupolar coupling constant (assuming the quadrupolar asymmetry parameter to be zero) were calculated as ca. 1.3 × 10^{-8} s and 0.5 MHz, respectively, from s_1 and s_2 and equations 57, 58, and 5 from the paper by Jaccard et al. (1986). The τ_c has the same order as the rotational correlation time of bovine serum albumin (10^{-8} s). As shown in Figure 3, the double-quantum relaxation of K in an albumin solution had only one relaxation rate constant (s_{dq} = 290 s^{-1}), which suggested the absence of exchange processes in this time scale (Jaccard et al., 1986). This value agreed with the value (180–250 s^{-1}) calculated from eq 12 of Rooney et al. (1988) using s_1 and s_2.

In living cells, it has been suggested that a spectrum consisting of the coexistence or superposition of inhomogeneous powder patterns and homogeneous biexponential spectra and/or spectra of intermediate nature (rapid exchange) may obtain [e.g., Springer (1988) and Civan and Shporer (1989)]. In this study, we observed two Lorentzian signals corresponding to the $|^1/_2><^{-1}/_2|$ coherence and the $|^{-1}/_2><^{-3}/_2|$, $|^3/_2><^1/_2|$ coherences of the intracellular K. The difference in resonance frequency between the two resonances was negligibly small. We may, therefore, exclude a pure inhomogeneous powder pattern model. When we assumed a single homogeneous population and used the values of s_1 and s_2, $\omega\tau_c$ was calculated to be ca. 2.7, the correlation time was ca. 2.5 × 10^{-8} s, and the quadrupolar coupling constant (assuming the quadrupolar asymmetry parameter to be zero) was ca. 1.4 MHz. Supporting evidence for this assumption is given as follows: (a) The estimated value of the quadrupolar coupling constant has a similar value estimated in some potassium complexes (1.1–2.6 MHz) (Das & Hahn, 1958; Drakenberg, 1986). When rapid exchange is operative, the apparent value might be an underestimate (Lerner & Torchia, 1986). (b) The T_1 values of intracellular K can be estimated from the values of τ_c and the quadrupolar coupling constant (Jaccard et al., 1986). Calculated values of ca. 2 and 6 ms are in reasonable agreement with the observed values of T_1 for the intracellular K. (c) The double-quantum relaxation of intracellular K also had a single relaxation rate, and the measured s_{dq} value lay within the range of values for s_{dq} calculated from s_1 and s_2. Thus, these results cannot eliminate the possibility of the rapid exchange process. Therefore, studies of the field and temperature dependencies of relaxation pa-

rameters are necessary to confirm this assumption (Lerner & Torchia, 1986).

ACKNOWLEDGMENTS

We thank H. Hattori, O. Ichikawa, A. Ikeda, K. Suzuki, and H. Ookawara for their technical assistance and Prof. M. M. Civan and Dr. M. C. Steward for helpful discussion.

Registry No. K, 7440-09-7.

REFERENCES

Bax, A., Freeman, R., & Kempsell, S. P. (1980) *J. Am. Chem. Soc. 102*, 4849-4851.

Chu, S. C., Pike, M. M., Fossel, E. T., Smith, T. W., Balschi, J. A., & Springer, C. S., Jr. (1984) *J. Magn. Reson. 56*, 33-47.

Civan, M. M., & Shporer, M. (1989) *Current Topics in Membranes and Transport*, Academic, New York (in press).

Das, T. P., & Hahn, E. L. (1958) *Solid State Physics*, Suppl. 1, pp 180-183, Academic, New York.

Drakenberg, T. (1986) *Annu. Rep. NMR Spectrosc. 18*, 231-283.

Heilbrunn, L. V. (1938) *An Outline of General Physiology*, p 62, Saunders, Philadelphia.

Jaccard, G., Wimperis, S., & Bodenhausen, G. (1986) *J. Chem. Phys. 85*, 6282-6293.

Lerner, L., & Torchia, D. A. (1986) *J. Am. Chem. Soc. 108*, 4264-4268.

Murakami, M., Seo, Y., Watari, H., Ueda, H., Hashimoto, T., & Tagawa, K. (1987) *Jpn. J. Physiol. 37*, 411-423.

Pekar, J., & Leigh, S. (1986) *J. Magn. Reson. 69*, 582-584.

Pekar, J., Renshaw, P. F., & Leigh, S. (1987) *J. Magn. Reson. 72*, 159-161.

Piantini, U., Sørensen, O. W., & Ernst, R. R. (1982) *J. Am. Chem. Soc. 104*, 6800-6801.

Rooney, W. D., Barbara, T. M., & Springer, C. S., Jr. (1988) *J. Am. Chem. Soc. 110*, 674-681.

Seo, Y., Murakami, M., Matsumoto, T., Nishikawa, H., & Watari, H. (1987a) *Pfluegers Arch. 409*, 343-348.

Seo, Y., Murakami, M., Suzuki, E., & Watari, H. (1987b) *J. Magn. Reson. 75*, 529-533.

Shaka, A. J., & Freeman, R. (1983) *J. Magn. Reson. 51*, 169-173.

Springer, C. S., Jr. (1987) *Annu. Rev. Biophys. Biophys. Chem. 16*, 375-399.

Energetics of Reserpine Binding and Occlusion by the Chromaffin Granule Biogenic Amine Transporter†

Gary Rudnick,*,‡ Sonia S. Steiner-Mordoch, Hana Fishkes, Yael Stern-Bach, and Shimon Schuldiner

Department of Molecular Biology, Hadassah Medical School, The Hebrew University of Jerusalem, Jerusalem, Israel

Received June 19, 1989; Revised Manuscript Received September 7, 1989

ABSTRACT: The energetics of reserpine binding to the bovine adrenal biogenic amine transporter suggest that H^+ ion translocation converts the transporter to a form which binds reserpine essentially irreversibly. Reserpine binding to bovine adrenal chromaffin granule membrane vesicles is accelerated by generation of a transmembrane pH difference (ΔpH) (interior acid) or electrical potential ($\Delta\psi$) (interior positive). Both components of the electrochemical H^+ potential ($\Delta\tilde{\mu}_{H^+}$) must be dissipated to block reserpine binding, and generation of either one stimulates the binding rate. Reserpine binding is less dependent than amine transport on the ΔpH, suggesting that translocation of fewer H^+ ions is required to expose the high-affinity site than are required for net transport. Bound reserpine dissociates very slowly, if at all, from the transporter. Binding is stable to 1% cholate, 1.5% Triton X-100, 1 M SCN^-, and 8 M urea, but sodium dodecyl sulfate (0.035%) and high temperatures (100 °C) released bound resperine, indicating that binding is noncovalent. The results raise the possibility that the transporter, by translocating one H^+ ion outward down its concentration gradient, is converted to a form that can either transport a neutral substrate molecule inward or occlude reserpine in a dead-end complex.

The biogenic amine transporter is responsible for accumulation of serotonin, dopamine, norepinephrine, epinephrine, and histamine within secretory vesicles in a variety of cells (Kanner & Schuldiner, 1987; Njus et al., 1986). The energy required for amine accumulation comes from an ATP-driven H^+ pump in the secretory vesicle membrane which acidifies the vesicle lumen (Rudnick, 1986a,b). The amine transporter exchanges intravesicular H^+ ions for cytoplasmic biogenic amines, thereby coupling the downhill flux of accumulated H^+ ions with uphill amine accumulation. Although the H^+/amine stoichiometry for this process has been known for many years (Knoth et al., 1981a; Johnson et al., 1981) and some kinetic aspects of the transport process have been described (Maron et al., 1983), many aspects of the mechanism have remained elusive. The ionic form of the substrate amine which binds to the transporter is not known. Although a variety of evidence suggests that each amine molecule exchanges in its uncharged form with one H^+ ion (Ramu et al., 1983; Scherman & Henry, 1981; Kobold et al., 1985), the possibility that the cationic species (which predominates at cytoplasmic pH) exchanges with two H^+ ions has never been ruled out (Knoth et al., 1981b; Daniels & Reinhard, 1988). Moreover, the mechanism by which H^+ and amine fluxes are coupled remains unknown.

† This work was supported by U.S. Public Health Service Grants HL-21217 (to G.R.) and NS-16708 (to S.S.).

‡ On sabbatical leave from the Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

0006-2960/90/0429-603$02.50/0