Spin-Lattice Relaxation Measurements Used in an Attempt at Detecting Complex Formation between Noradrenaline and Lithium in Aqueous Solution at Physiological pH and Temperature

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The longitudinal relaxation times of $^7$Li and $^{13}$C have been measured in aqueous solutions of $\alpha$-noradrenaline and lithium chloride by the FIRFT method with correction for the effect of inhomogeneities in the RF field. The results indicate negligible complex-formation between $Li^+$ and noradrenaline at physiological pH and temperature.

Lithium salts have for long been one of the main agents used in the treatment of mania but the biochemical basis of its therapeutic effect remains undetermined. One way in which lithium may affect the nervous system is by influencing the release or uptake of noradrenaline $^{1,2}$ It would be of interest, therefore, to determine whether or not there is complex-formation between lithium ions and noradrenaline in aqueous solution at physiological pH and temperature.

As pointed out by James and Noggle $^2$ the longitudinal relaxation-rate of quadrupolar nuclei such as $^{23}$Na is very sensitive to the electronic environment around and hence complexation of the ion. This is because relaxation is due predominantly to the interaction of the quadrupole moment of the nucleus with the electric field-gradient at the nucleus and the size of the latter depends heavily on the symmetry of the complex. The quadrupolar relaxation-rate is also directly proportional to the correlation-time of the nucleus and is likely to be increased on complexation of the ion with a larger molecule. Thus, Forsén et al. $^3$ used $^{23}$Na $T_1$ measurements to study the weak complexation of sodium ions by sugars in aqueous solution. Relaxation of $^7$Li is not always dominated by quadrupolar relaxation, but relaxation seems nevertheless to be quite sensitive to complexation. Jagur-Grodzinski et al.$^4$ measured a formation constant of 1.1 M$^{-1}$ for complexation of $Li^+$ with $N,N'$-diheptyl-$N,N'$-tetramethyl-3,7-dioxanonanediamide (NDA) in pyridine solution, and Plausch et al.$^5$ have studied the complexation of nucleosides, nucleic acid bases and ribose in DMSO solution using this method. Yokono et al.$^6$ have studied the interaction of $^7$Li$^+$ with nucleotides in aqueous solution and reported significant increase in the relaxation rate of $^7$Li$^+$.

Quadrupolar relaxation is responsible for about 44% of the relaxation rate of $^7$Li$^+$ in aqueous solution at ambient temperature, the other 56% being due to dipole–dipole relaxation.$^7$ The electric field-gradient at the $Li^+$ nucleus in dilute solution is expected to be small because of the tetrahedral arrangement of the solvating water molecules.$^8$ Complexation $via$, for example, the catechol hydroxyl groups of noradrenaline with displacement of water molecules from the inner coordination-shell of $Li^+$ would greatly decrease the symmetry around the ion and one could anticipate a correspondingly increased relaxation rate. If the coordination was, on the other hand, outer-sphere, the electric field-gradient might not be greatly enhanced, but the relaxation rate would nevertheless be expected to increase due to an increase in the correlation-time.

In the latter case one might also expect to observe some increase in the relaxation rate of the $^{13}$C nuclei of noradrenaline, especially if a 2:1 complex (noradrenaline:$Li^+$) was formed.

Preparation of solutions. All glassware was freed from possible contamination by paramagnetic ions by being soaked overnight in basic EDTA solution followed by being extensively washed with deionised water. Solutions containing the desired concentrations of lithium chloride (Merck, p.a.) and $\alpha$-n-
noradrenaline hydrochloride (Fluka, purum) were prepared in a nitrogen atmosphere, using nitrogen-degassed solvents and solutions, and sealed under nitrogen into the sample tubes. The pH of the solutions were measured using a pH-meter with a combined glass-calomel electrode, pH = 7 was approximated by adjusting to a pH-meter reading of 6.6. Adjustments were made by the addition of small quantities of LiOH solution in the case of H2O solutions and of NaOD solution in the case of D2O solutions. The final [Na+] of the latter solutions did not exceed 0.01 M compared with an [Li+] of 2 M.

Measurement and calculation of $T_1$. $T_1$ measurements were made using a JEOL FX-100 spectrometer operating at a frequency of 25.05 MHz with proton noise-decoupling for $^{13}$C and at a frequency of 38.71 MHz for $^7$Li. Measurements on $^{13}$C were performed on D2O solutions using 5 mm tubes filled to a depth of 2.6 cm. Measurements on $^7$Li were performed in H2O solution. In the latter case, the samples were contained in spherical cells of approximately 6 mm diameter inserted into 10 mm tubes containing a few ml's of D2O as lock-substance, see Fig. 1.

The Fast Inversion-Recovery Fourier Transform (FIRFT) technique,10 which uses the pulse-sequence (-180°- $t_1$- 90°- Acquire - Pulse-delay - $\lambda$), was used for measuring $T_1$. The FIRFT experiment is optimally efficient when the waiting time ($W$ = Pulse-delay + Acquisition-time) is made to be $2T_B^*$ where $T_B^*$ is the longest $T_1$ to be measured in a given experiment.11 This optimal value of $T_B^*$ was used in all of the $^7$Li measurements; however, $W$ = $2.5T_B^*$ and $W$ = $T_B^*$ were used in the two $^{13}$C experiments. $T_1$ was calculated by a non-linear least-squares fit12 of the data to eqn. (1): $M_i = M_o \left[1 - \{1 + I \left[1 - \exp(-W/T_1)\right]\} \exp(-t_i/T_1)\right]^{13}$ (1) In this equation $I$ is a $B_i$-inhomogeneity parameter whose input value is calculated as described in Ref. 13 from relative peak-intensities obtained after the application of 90 and 270° pulses. $M_o$ and $T_1$ were treated as variable- and $I$ and $W$ as fixed-parameters in the fitting procedure. At least eight different values of $t_i$ were used in each determination and the resulting $T_1$ values given in Tables 1 and 2 are the result of at least one determination on at least two separately-prepared samples. Inaccuracies in the measured $T_1$ values caused by inhomogeneities in the radio-frequency field ($B_i$) become more serious as $W$ is decreased.13 This problem could be decreased in the case of $^7$Li measurements by using a small spherical sample placed centrally in the receiver coil (Fig. 1). However, the use of small samples for the $^{13}$C measurements would have necessitated much longer experimental times due to the low concentrations of noradrenaline achievable. The use of eqn. (1) takes into account the $B_i$-inhomogeneity by the application of an inhomogeneity parameter $I$ which can be measured experimentally. We have tested the use of eqn. (1) to calculate $T_1$ on data obtained from normal-sized (2.6 cm depth in 5 mm tubes) samples in our NMR instrument. Undegassed samples containing (by volume) 80% cyclohexanol and 20% C6D6 were used. Data for the calculation of the $^{13}$C $T_1$'s of cyclohexanol were obtained using the FIRFT sequence with various values of $W$. $T_1$ values were then calculated using a non-linear least-squares fit to eqn. (1) at

![Fig. 1. Sample-arrangement for $T_1$ measurements on $^7$Li.](image)

![Fig. 2. Plot of calculated $T_1$ values from eqn. (1) against waiting-time $W$ using different input values of inhomogeneity parameter $I$.](image)

$\triangle$, $I=0.7$; ○, $I=0.77$; ▲, $I=0.8$; ▽, $I=0.9$; ○, $I=1.0$. Experimental data are from C-3,5 in cyclohexanol.

various input values of the inhomogeneity parameter $I$. The inhomogeneity parameter was also measured experimentally. Results for the C-3,5 in cyclohexanol are plotted in Fig. 2. As can be seen, the calculated $T_1$ values are relatively constant with change in $W$ when the input $I$ lies between 0.77 and 0.80 agreeing well with the measured value of $I$ which was 0.79 (0.02). Larger and smaller values of $I$ cause increasing dependency of $T_1(\text{calculated})$ on $W$. Special attention is drawn to the large variation in $T_1(\text{calculated})$ when $I$ is given the value of 1, corresponding to the situation when results are calculated without taking into account $B_1$-inhomogeneity. It is also worth noting that even when $W=5T_1$ (inversion-recovery conditions), the $T_1$ values calculated without taking $B_1$-inhomogeneity into account are about 17% lower than those calculated taking it into account. That the results obtained when $B_1$-inhomogeneity is taken into account is more correct, is supported by the data in Fig. 3, which is a plot of $M_0$ determined experimentally, divided by $M_0$ calculated from eqn. (1), against $W$. $M_0(\text{experimental})$ was obtained by including a pulse-interval ($t_p$) of approximately $5T_1$ in the experiments. The $M_0$ (calc.) values are closest to $M_0$ (exp.) when $I$ is the experimentally measured value.

Measurement of $I$ on each of the noradrenaline-containing samples was impractical due to the very long experimental times which would have been required. Instead a value of $I=0.80$ (0.02) measured on a 2.6 cm deep sample of 0.6 M sucrose in $D_2O$ was used in the calculation of $T_1$ from noradrenaline.$^{13}$C data. $I$ was also measured on a 0.6 M sucrose solution containing 2 M LiCl to ensure that the high salt-concentration did not alter $I$. The result was the same within experimental error. $B_1$-inhomogeneity was also taken into account in calculating $^7$Li data, but here, $I$ was measured on the relevant sample.

The probe-temperature was controlled by a JEOL temperature-control unit at 38°C (calibration after every experiment by the use of methanol and ethylene glycol samples) and at least 20 min were allowed for thermal equilibration of the samples before measurements were begun. In order to avoid variations in temperature between pairs of samples (e.g. $^7$Li $T_1$ measured with and without noradrenaline) due to possible inaccuracies in resetting the temperature-control unit, the second of a pair was always run directly after the first without readjustment of the temperature.

**Discussion.** Our suggestion that noradrenaline (I) might be complexed by lithium ions, stems from the observation that this catecholamine contains four potential ligand groups. As the predominant form of noradrenaline at pH 7 is likely to be the cation (2),$^{14,15}$ complex formation at the side-chain might be disfavoured, but this still leaves the possibility of complexation by the two aromatic hydroxyl groups. Complexation could result in either the 1:1 species (3) or the 2:1 species (4). The formation of (3) would be reflected in a powerful decrease in the $^7$Li $T_1$ due to both an increase in correlation time and an increase in the electric field-gradient at the metal nucleus. Formation of (4) would result in a decreased $^7$Li $T_1$ due to the increase in correlation-time of the complex over that of the free ion; furthermore, there should be a decrease in the $^{13}$C $T_1$ due to an increased correlation time of a 2:1 complex over that of the uncomplexed molecule. By consideration of the results of Jagur-Grodzinski et al.$^4$ we estimate that the formation of complexes with formation constants of around $10^{-1}$ M or

<table>
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<th>[Noradrenaline]/M</th>
<th>$T_1$/s$^a$</th>
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<tr>
<td>0.0</td>
<td>23.6</td>
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<td>0.2</td>
<td>22.3</td>
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$^a \pm 10\%$. 

Table 1. $^7$Li longitudinal relaxation times. [Li$^+$] = 0.02 M, $H_2O$ solution pH7, 38°C.
Table 2. $^{13}$C longitudinal relaxation times. [Noradrenaline] = 0.2 M, D$_2$O solution pD7, 38°C.

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>$T_1$/$^b$ (Li$^+$ = 0.0 M)</th>
<th>$T_1$ (Li$^+$ = 2.0 M)</th>
<th>$T_1(-\text{Li}^+)/T_1(+\text{Li}^+)$</th>
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</thead>
<tbody>
<tr>
<td>2</td>
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<tr>
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<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>8</td>
<td>0.9</td>
<td>0.7</td>
<td>1.3</td>
</tr>
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*a* Assignments according to Ref. 14. $^b$ ± 12%.

less should be possible by application of $T_1$ measurements to this system.

The results of our studies are given in Tables 1 and 2. Our observation that there is no significant decrease in $T_1$ either for $^7$Li, in the presence of a 10-fold molar excess of noradrenaline, or for $^{13}$C of noradrenaline in the presence of a 10-fold molar excess of lithium ions, means that the formation of either (3) or (4) does not occur to any significant extent in aqueous solution at physiological temperature and pH. The ratio of $T_1$ in the absence of Li$^+$ to $T_1$ in the presence of Li$^+$ of approximately 1 for all measured carbons, is no more than expected from the difference in viscosity of these two solutions ($\eta$ 2MLiCl/$\eta$ H$_2$O at 25°C = 1.2). Our results would also seem to rule out the possibility of complex-formation between lithium and the other neurochemically important catecholamines dopamine and adrenaline as these have similar or worse complexing possibilities than noradrenaline.

REFERENCES


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