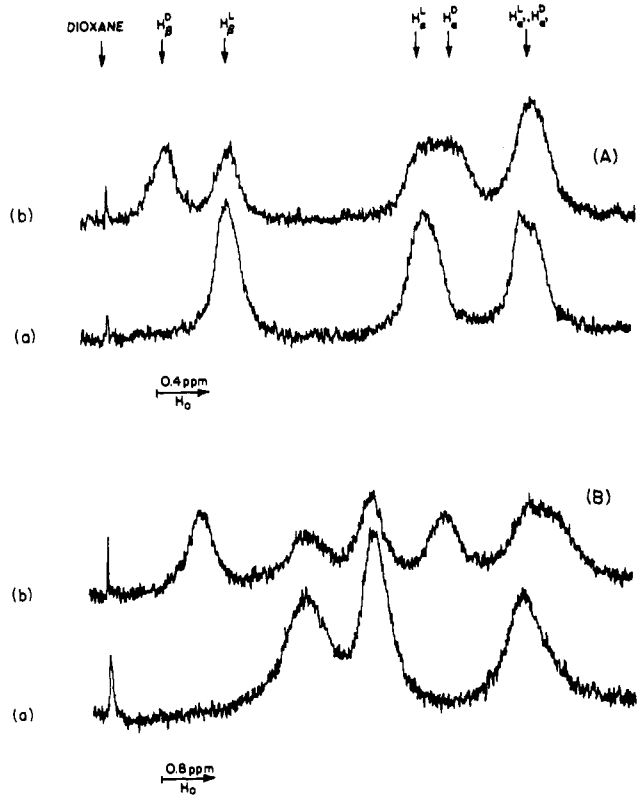

Figure 2. Proton magnetic resonance spectra at 90 MHz of aqueous (D_2O) solutions at pD 6.4 containing 0.1 M CoATP and (a) 0.17 M L-norepinephrine or (b) 0.17 M racemic norepinephrine at 27 °C (A) and 0 °C (B). The sharp peak on the left is due to a trace of *p*-dioxane used as an internal reference.

$1/T_1$, being the longitudinal relaxation time.[5] The corresponding distance in the complex of D-norepinephrine is 5.4 Å as calculated using a similar relationship for the transverse relaxation rate.[7] The distances measured on molecular models based on the structures given in Figure 1 are 5.2 and 6.0 Å for the L and D enantiomers, respectively. Bearing in mind that distances derived from NMR data are averages taken over intramolecular rotations that are rapid on the NMR time scale, the agreement between the two sets of distances should be regarded as good. Thus we have good evidence for the preferred conformations of the ethanolamine side chains of L- and D-norepinephrine in their complexes with ATP as depicted in Figure 1.

The results presented here demonstrate that CoATP can be used to resolve NMR spectra of racemic mixtures in aqueous solution; i.e., it can serve as an aqueous chiral shift reagent. In this way the different disposition of the β-hydroxyl group (and the β proton) in L- and D-norepinephrine in their complexes with CoATP has been established. In the complex with ATP or with its metal ion chelate all of the functional groups of L-norepinephrine (the α-ammonium, the β-hydroxyl, and the catechol hydroxyls) are positioned on the same side (cf. Figure 1). This arrangement is impaired for D-norepinephrine in which the β-hydroxyl faces the opposite side. It has been suggested that for maximum physiological effect the attachment to the receptor site must involve three groups: the α-amino, the β-hydroxyl, and the aromatic moiety.[8] Our findings may form a conformational basis for rationalization of the different biological activities of the norepinephrine enantiomers provided that a ternary norepinephrine-ATP-receptor complex is formed.[9]

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Norepinephrine Complexes and Reduces Vanadium(V) to Reverse Vanadate Inhibition of the (Na,K)-ATPase

Sir:

Recently, it has been shown that vanadium, which is essential to mammalian life, may be present in muscle tissue at concentrations sufficient to inhibit the sodium and potassium stimulated adenosine triphosphatase ((Na,K)-ATPase).[1] There are several reports of catecholamine activation of (Na,K)-ATPase in vitro,[2] and it has been proposed that these compounds simply reverse vanadate inhibition.[2d] In this communication we present evidence showing that the reversal (and several other related effects) can be explained by a series of complexation and redox reactions between V(V) and norepinephrine (NE).

Catechol and its derivatives react rapidly with VO_2^+ in high acid[3] and with vanadate at physiological pH (i.e., pH 6-8).[4] With excess vanadate, the blue color characteristic of VO^{2+} appears instantaneously upon mixing. At low [V(V)] to [catechol] ratios a yellow color is observed, which upon standing turns brown. The blue color of solutions with initial excess vanadate gradually disappears, resulting in the same brown solution. Both processes require a few minutes, depending on concentration and the catechol derivative; the blue color remains in solutions kept under argon.

The initially observed reaction has been interpreted as a formal redox reaction: 2V(V) + catechol = 2V(IV) + *o*-quinone + $2H^+$.[3,4] The subsequent disappearance of the blue V(IV) produced in the reaction is most likely due to reoxidation by atmospheric oxygen to V(V), a rapid process.[5] The slower conversion of the yellow color to brown can be explained by the well-known polymerization of the organic quinonoid product.[6] This interpretation can be applied to the interaction between vanadate and norepinephrine, a catechol derivative, explaining the reversal of (Na,K)-ATPase inhibition by vanadate.

Spectral studies show that upon mixing vanadate with NE, two new peaks form rapidly: one at 680 nm assigned to the coordinated vanadyl (VO^{2+}) center and one at 295 nm assigned to coordinated ligand. At longer times there is a simultaneous appearance of peaks at 300 and 485 nm (probably a hydroxyquinone). The rate of appearance of the latter peaks may be impeded with dithiothreitol. The addition of MnO_4^-

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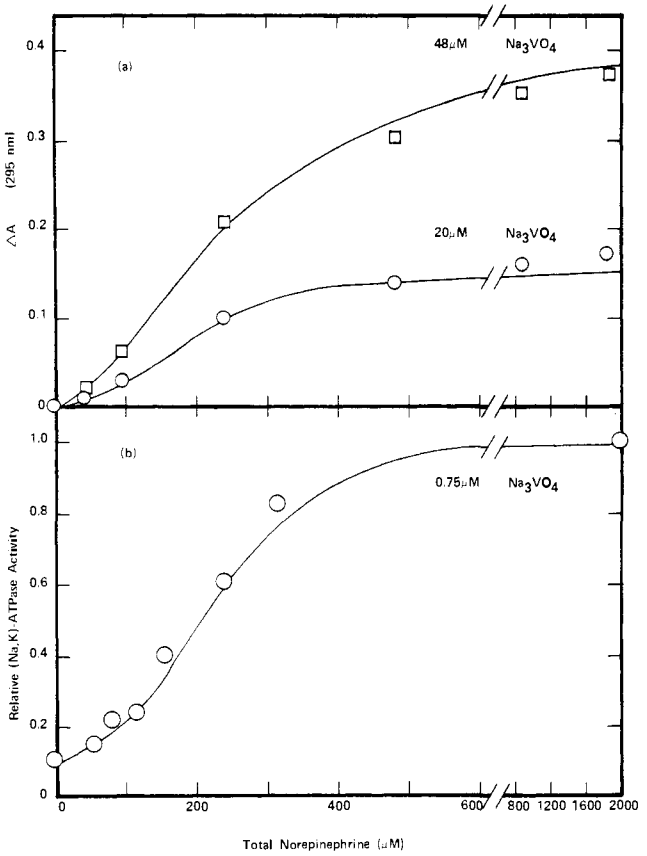

Figure 1. (a) A difference titration of Na_3VO_4 (Fisher Scientific) with L-norepinephrine (Sigma Chemical Co.). Partitioned cuvettes were used with 0.44-cm light path per compartment. The norepinephrine and Na_3VO_4 were added to the same compartment in the sample cuvette but to separate compartments in the reference cuvette. Corrections were made for dilution effects (<5%). The buffer contained 20 mM Tris·Cl, 20 mM $MgCl_2$, 20 mM KCl, 100 mM NaCl, and 1 mM dithiothreitol at pH 7.4, 25 °C, and the absorbance (ΔA) was measured at 295 nm 2 min after mixing. The solid lines were calculated using eq 1-3 and the parameters in the text. (b) The relative activity of dog kidney (Na,K)-ATPase at steady state in the presence of 0.75 μM Na_3VO_4 vs. the L-norepinephrine concentration in the assay. The enzyme was prepared and assayed as previously described[2d] in the presence of 20 mM $MgCl_2$, 20 mM KCl, 100 mM NaCl, 5 mM ATP (vanadate free), and 1 mM dithiothreitol at pH 7.4, 37 °C. The enzyme activity in the absence of vanadate was assigned the value 1 and this activity was unaffected by norepinephrine. The steady-state activity was independent of the order of addition of enzyme, norepinephrine and vanadate.

or IO_4^- to NE causes simultaneous appearance of the 300- and 485-nm peaks but not the 295- and 680-nm peaks.

A spectrophotometric titration of vanadate by NE is shown in Figure 1a. This result can be interpreted by assuming that the change in absorbance is due to the formation of a bis-V(IV) complex, i.e., a complex of formula $V(IV)L_2$ where L is NE. The difference in absorbance at 295 nm, ΔA, is then given by the expression

$$\Delta A = \frac{a_1[V]_T}{1 + K_2[NE] + 1/K_1[NE]} + \frac{a_2[V]_T}{1 + 1/K_2[NE] + 1/K_1K_2[NE]^2} \quad (1)$$

where $[V]_T$ is the total vanadium added as Na_3VO_4, K_1 and K_2 are the mono and bis complex stability constants, a_1 and a_2 are absorptivity coefficients for the mono and bis complexes, and [NE] represents the free norepinephrine concentration. This equation was fit to the data in Figure 1a by an iterative nonlinear least-squares procedure with [NE] estimated from the total norepinephrine $[NE]_T$ and total vanadate concentration $[V]_T$ using

$$[NE] = \frac{-(1 + K_1[V]) + [(1 + K_1[V])^2 + 8K_1K_2[V][NE]_T]^{1/2}}{4K_1K_2[V]} \quad (2)$$

$$[V] = [V]_T/(1 + K_1[NE] + K_1K_2[NE]^2) \quad (3)$$

The best fit was obtained with $K_1 = 2.8 \times 10^2\ M^{-1}$, $K_2 = 9.5 \times 10^4\ M^{-1}$, and $a_1 = \frac{1}{2}a_2 = 9.47 \times 10^3\ M^{-1}\ cm^{-1}$.

The similarity between the titration curves for NE reversal of vanadate inhibited (Na,K)-ATPase activity (Figure 1b) and $V(IV)L_2$ complex formation (Figure 1a) argues that NE acts by lowering the free vanadate concentration in the assay.

Transient spectroscopy by stopped-flow technique has been carried out at 400 nm on vanadate-NE reaction mixtures; representative traces are shown in Figure 2. Only the $(\overline{NE})$ dependence could be studied because of polymer formation in solutions of excess vanadate. In terms of chemical relaxation, the fastest effect depends on $[\overline{NE}]$,[2] saturating at high $[NE]_T$; the middle and slowest effects depend on $[\overline{NE}]$; the overbar denotes final concentration.

If the first transient effect is complexation according to $V + 2NE \rightleftarrows [V(NE)] + NE \rightleftarrows V(NE)_2$ with [V(NE)] in a steady-state concentration, then[7]

$$k_{obsd} = \frac{k_1k_2(4[\overline{V}][\overline{NE}] + [\overline{NE}]^2) + k_{-1}k_{-2}}{k_{-1} + k_2[\overline{NE}]} \quad (4)$$

where k_1, k_{-1}, k_2, and k_{-2} are the forward and reverse rate constants ($M^{-1}\ s^{-1}$) for the mono and bis complexations, respectively. Under the conditions $k_{-1} > k_2[\overline{NE}]$, eq 4 shows limiting $[\overline{NE}]^2$ dependence at low $[\overline{NE}]$ which saturates to a $[\overline{NE}]$ dependence as the condition $k_{-1} < k_2[\overline{NE}]$ is met with increasing $[\overline{NE}]$.

To explain the second transient, we assume that norepinephrine is oxidized by the vanadate complex; $V(NE)_2 + NE \rightleftarrows V(IV) + Q^{-}\cdot$, where V(IV) is one or more complexes and $Q^{-}\cdot$ is a semiquinone. Then the specific rate is

$$(1/[Q^{-}\cdot])d(\Delta[Q^{-}\cdot])/dt = k_3([\overline{V(NE)_2}] + [\overline{NE}]) + k_{-3}([V(IV)] + [\overline{Q^{-}\cdot}]) \simeq k_3[\overline{NE}] \quad (5)$$

In eq 5, k_3 and k_{-3} are forward and reverse rate constants,

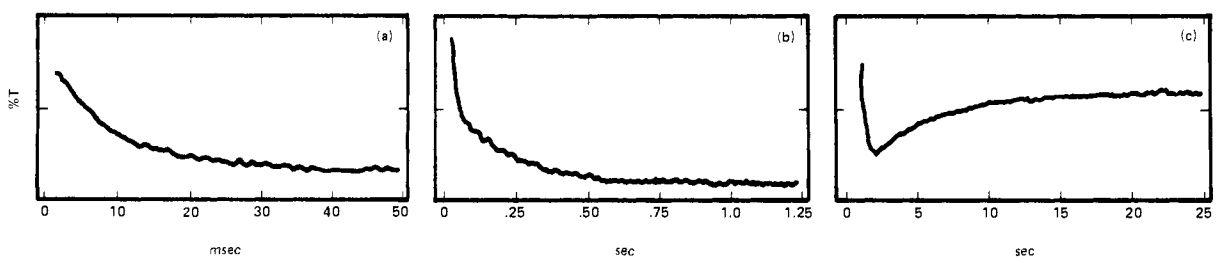

Figure 2. Representative oscilloscope traces of the NE-V(V) reaction. The vertical axis is the photomultiplier voltage which is linearly proportional to the relative transmittance of the solution. Conditions $[NE]_T = 7.4 \times 10^{-4}$ M, $[V(V)]_T = 2.75 \times 10^{-5}$ M, pH 8.62: (a) 0.1 V full scale, 50-ms elapsed time; (b) 0.05 V full scale, 1.25-s elapsed time; (c) 0.05 V full scale, 25-s elapsed time. The long wavelength tail of the 295 absorbance peak was monitored at 400 nm.

respectively; we have assumed that the back-reaction is unimportant, and $[\overline{NE}] > [\overline{V(NE)_2}]$.

The longest transient can be ascribed to further reactions of the quinone products of the redox reaction. These reactions include semiquinone quenching, oxidative addition of hydroxide to the quinone, and other processes.[8]

Reversal of the vanadate inhibition of (Na,K)-ATPase is accomplished by norepinephrine and other catecholamines through complexation and reduction of vanadate. These compounds are more effective in removing free vanadate than other chelators (e.g., EDTA) under physiological conditions because they do not readily form competing complexes with Mg^{2+} and Ca^{2+}.[9] Thus, the model presented here is consistent with the data, and explains several other related observations.

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Correlated Rotation of *tert*-Butyl Groups in Tri-*tert*-butylsilane

Sir:

In crowded systems, the correlated (coupled) rotation of two or more alkyl groups may become energetically more feasible than the independent rotation of a single group.[1] In view of current interest in "gearing" or "cogging" effects of alkyl groups,[2-5] we now wish to report evidence based on empirical force field (EFF) calculations[6] and DNMR studies that the rotation of the *tert*-butyl groups in tri-*tert*-butylsilane (**1**) is correlated.

According to full relaxation EFF calculations, the ground state of **1** has C_3 symmetry, with torsional angles (ϕ = C_m–C_q–Si–H)[8] of 43°.[9] To study dynamic processes in **1**, we resorted to incremental group driving calculations.[14] Using this method, strain energies of **1** were calculated[6] with one C_m–C_q–Si–H torsional angle frozen at successively increasing or decreasing values,[15] while all other internal parameters were allowed full relaxation. When one *tert*-butyl group of **1** was driven through a staggered (*S*) conformation, a barrier of 5.1 kcal/mol was surmounted as the other two (unfrozen) *tert*-butyl groups responded by rotating through staggered conformations, resulting in overall enantiomerization by the *SSS* pathway (Figure 1). When the *tert*-butyl group was driven through the eclipsed (*E*) conformation, the other two groups again responded by rotating through staggered conformations (*ESS*, Figure 1), with a calculated barrier of 6.8 kcal/mol. These two processes represent correlated rotations, in the following sense.

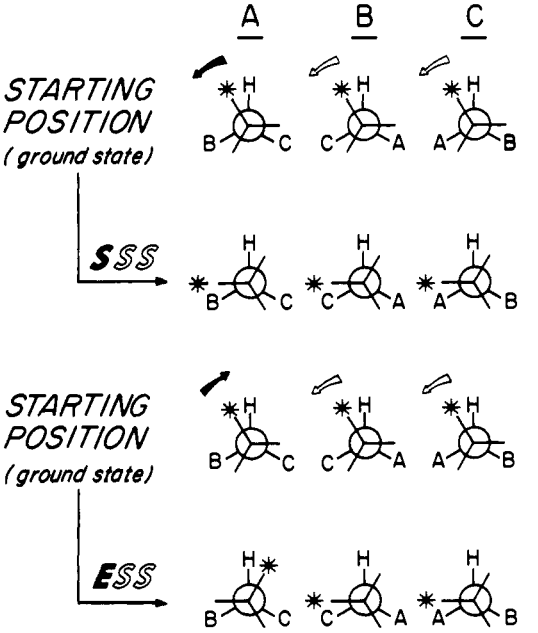

Figure 1. Two torsional pathways for *t*-Bu_3SiH (see text). The view down the C_q–Si bond axis from C_q to Si is shown for each of the three *tert*-butyl groups (A, B, and C, as denoted by the column heading). One methyl in each *tert*-butyl group is marked with a star to provide a point of reference. Solid arrows indicate the direction in which group A is driven; hollow arrows indicate the overall direction of rotation induced in the other two groups.

The possible torsional pathways which effect topomerization or enantiomerization can be delineated by means of group theoretic techniques.[16] Considering only rotations about the *t*-Bu–Si bonds,[17] the C_3 point group partitions the nonrigid molecular symmetry group of feasible rearrangements[18] (G_{162} = $(C_3)^3 \wr C_{3v}$) into sixteen distinct rearrangement modes: ten enantiomerizations (two of which are represented by the *SSS* and *ESS* processes), five topomerizations, and the identity mode. We consider rotation to be correlated if, and only if, a single-step process leads to permutational rearrangement (site exchange) of the methyl groups in more than one of the three *tert*-butyl groups. It follows that all enantiomerizations, including the two processes depicted in Figure 1, involve correlated rotations.

In the threshold (*SSS*) mechanism, all three *tert*-butyl groups undergo net conrotation (see Figure 1). Two of the three diastereotopic methyl sites are thus rendered equivalent (i.e., the average symmetry is C_{3v}, with the unique methyl group anti to Si–H). In the *ESS* mechanism, two of the three pairwise interactions between *tert*-butyl groups involve net disrotation; the third interaction involves net conrotation. By this mechanism, all three methyl sites are equivalenced by successive rearrangements in which a given *tert*-butyl group passes through *S* and *E* conformations, e.g., by a succession of the

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