Lithium and rubidium: Effects on the rhythmic swimming movement of jellyfish *(Aurelia aurita)*

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Summary. The effects of adding LiCl, RbCl, KCl or NaCl to sea water at concentrations up to 30 mmoles/l on the frequency of contraction of jellyfish *(Aurelia aurita)* suggest that studies on phylogenetically low animals with relatively simple nervous systems may be of use to determine mechanisms of action of lithium and rubidium on movements.

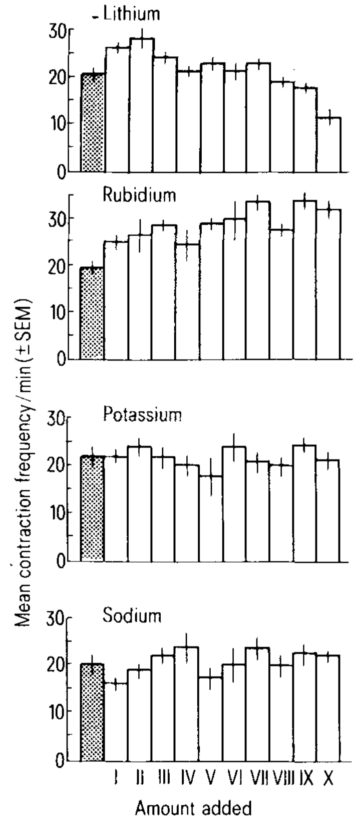
Current interest in effects of lithium and rubidium on biological processes stems mainly from the use of these 2 alkali metal cations in the treatment of mental disorders. Lithium is used primarily to treat bipolar manic-depressive illness, while rubidium is used on an experimental basis in the treatment of depression[2-4]. The rationale for the use of lithium and rubidium in psychiatry comes almost exclusively from studies carried out on guinea-pigs, rats, mice and monkeys[5-8]. The mechanisms responsible for the effects of lithium and rubidium on behaviour are still uncertain, however, perhaps partly because the complexity of the nervous system in these animals obscures the relationship between neuronal events and behaviour.

Studies on effects of lithium and rubidium on behaviour in animals with a simple nervous system may help clarify their mechanisms of action. The organization of the nervous system in jellyfish *(Aurelia aurita)* is as simple as can be found in the animal kingdom[9]. It consists of 1 nerve net of giant fibres and marginal ganglia which coordinate the rhythmic swimming movement, and another nerve net of smaller fibres which act on the marginal ganglia to excite or inhibit pacemakers[10,11]. The simple organization of the nervous system, the direct relationship between neuronal activity and movement, and the rhythmicity of the movement led us to determine whether lithium and rubidium alter the frequency of contraction of jellyfish.

Materials and methods. We carried out experiments during July and August, 1978. We used jellyfish *(Aurelia aurita)* ranging in diameter from 7 to 15 cm (weight 25–112 g) captured in a net on the day of experiments in Ebeltoft Bay near Femmøller, Denmark. We transported the jellyfish in sea water to Mols Laboratory, Strandkjaer, Denmark, and placed the animals singly or in pairs in a 4-l beaker (18 cm diameter) containing 2 l sea water maintained at 17 ± 1 °C. We determined the concentrations of sodium, potassium, calcium and lithium in the sea water and found them to be 228 mmoles/l, 5 mmoles/l, 6 mmoles/l and undetectable, respectively, at the start of experiments. Air was bubbled gently into the sea water near the surface. Jellyfish that showed an average of 10–30 uniform contractions per min under baseline conditions were selected for experiments. 61 of the ca. 110 jellyfish captured met this criterion. After baseline recording, solutions of NaCl, KCl, LiCl or RbCl at concentrations of 260 mmoles/l or 520 mmoles/l were added automatically by an infusion pump at a rate of from 0.05 to 3 ml/min to the sea water for up to 2 h. The number of contractions shown by each jellyfish was measured for 1 min every 15 min for up to 2 h. Analysis of variance was used to determine the statistical significance of the results.

Results. The figure shows the effects of adding lithium, rubidium, potassium or sodium to sea water on the frequency of contraction of jellyfish. Inspection of the data shows that the frequency tended to increase as the concentration of lithium increased up to 6 mmoles/l, and as the concentration of rubidium increased up to 30 mmoles/l. At concentrations of lithium above 6 mmoles/l, the frequency of contraction of the jellyfish tended to decrease. Addition of potassium or sodium to sea water at concentrations of up to 30 mmoles/l was without consistent effects on the frequency of contraction of the jellyfish.

Statistical analysis of the data showed that the overall effects of the salt treatments (main salt effect) on the frequency of contraction was significant ($p < 0.01$). Comparisons between pairs of salt treatments for their effects on the frequency of contraction failed to show significant differences, however, although the differences between lithium and rubidium ($F = 67.6$, $df = 1/253$) and between rubidium and sodium ($F = 63.2$, $df = 1/176$) just failed to attain significance at the 5% level. The overall effects of the amount of salt added to the sea water (main concentration effect) affected the frequency of contraction significantly ($p < 0.025$); the concentration effects for lithium and rubidium were significant ($p < 0.001$ and 0.005, respectively)



Effect of adding lithium, rubidium, potassium or sodium to sea water on the frequency of contraction of jellyfish *(Aurelia aurita)*. The frequency of contraction was first recorded under baseline conditions (stippled bars). Solutions of LiCl, RbCl, KCl or NaCl were then added to the sea water for up to 2 h, during which time the frequencT of contraction of each jellyfish was recorded for 1 min every 15 min. The amount of lithium, rubidium, potassium and sodium added to the sea water, in mmoles/l, was 1–3 (I), 4–6 (II), 7–9 (III), 10–12 (IV), 13–15 (V), 16–18 (VI), 19–21 (VII), 22–24 (VIII), 25–27 (IX) and 28–30 (X). Each bar corresponds to the mean ±SEM for 5–27 observations.

while the concentration effects for potassium and sodium were not statistically significant. The overall combined effects of salt and concentration (salt × concentration interaction) on the frequency of contraction was also significant ($p < 0.001$), and significant pairwise salt × concentration interactions were found for lithium and rubidium ($p < 0.001$), lithium and potassium ($p < 0.025$) and lithium and sodium ($p < 0.005$). The salt × concentration interaction for rubidium and sodium ($F = 2.3$, $df = 10/176$) just failed to attain significance at the 5% level, while the interactions for rubidium and potassium and for sodium and potassium were not statistically significant.

Discussion. Our findings show that addition of lithium or rubidium to sea water influenced the frequency of contraction of jellyfish. It is noteworthy that the changes in the frequency of contraction occurred without noticeable changes in the rate, evenness and uniformity of individual contractions. These observations suggest that lithium and rubidium affected mainly the rate of firing of the pacemakers in the marginal ganglia responsible for the rhythm of swimming movement[10-12]. It is of interest therefore to consider the actions of lithium and rubidium on ganglionic transmission.

The most consistent effect of lithium on ganglia is impairment of transmission, due primarily to lithium-induced depolarization of postsynaptic membranes[13-17]. While this action may be able to account for the decline in the frequency of contraction seen at lithium concentrations above 6 mmoles/l, it probably cannot explain the rise in the frequency of contraction seen at lower lithium concentrations. Thus, other effects of lithium on synaptic transmission may be involved in the effects of low concentrations of lithium on the frequency of contraction of jellyfish.

Rubidium is thought to act on ganglionic transmission by prolonging presynaptic action potentials, perhaps due to rubidium-induced hyperpolarization of synaptic membranes[8]. Prolongation of action potentials by rubidium could enhance neurotransmission, increase the rate of firing of pacemakers, and increase the frequency of contraction of jellyfish.

Electrophysiological studies clearly are needed to determine whether these explanations for the effects of lithium and rubidium on the swimming rhythm of jellyfish are correct.

In conclusion, a word should be said on the notion that lithium and rubidium can be expected to have opposite effects on biological processes[7,8,18]. We agree with those who consider there to be as yet too little evidence to support such an expectation in general[19]. It is to be noted, nevertheless, that lithium and rubidium, at concentrations between 6 and 30 mmoles/l, had opposite effects on the frequency of contraction of jellyfish in the present experiment. This observation suggests that studies on phylogenetically low animals, such as jellyfish, may be of use to determine relationships between neurophysiological and behavioural actions of lithium and rubidium.

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Hydrogen bonding by the sulphydryl group of glutathione

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Summary. The occurrence of hydrogen bond in the sulphydryl group of glutathione was investigated by means of Raman Spectroscopy. Evidence is obtained that SH group is free and H-bonding does not occur.

Hydrogen-bonding properties of OH and NH groups are well documented and established; on the contrary, the existence of SH--X bond is still controversial, at least in biological thiols[1-3]. In glutathione (GSH) the formation of intramolecular H-bond, involving the sulphydryl group, has been suggested[4-6] for explaining the reactive behaviour of -SH (for instance, the effect of hydrogen bond-breaking reagents, such as urea, on the reaction rate with AG^+ or nitroprusside, or the formation of a thiazolidine ring in strongly acidic solutions), but direct evidence is lacking.

The frequency of SH stretching vibration ν(SH), shifting to lower wavenumbers upon H-bond formation, is a sensitive test of the occurrence of hydrogen bonding: it is therefore worthwile to examine carefully by vibrational (Raman) spectroscopy the ν(SH) region of GSH. Raman spectra were taken with a Jarrell-Ash Raman Spectrometer, using an Ar^+ laser (4880 Å line) as excitation source and with a spectral resolution of 1.5 cm^{-1}.

In the solid state, the ν(SH) frequency is surprisingly low (2530 cm^{-1}) as compared with those of other simple thiols (see table 1), and such a value suggest, intra- or intermolecular H-bond. X-ray structure of GSH[7], however, does