rodents. More, PRANGE et al.[26] have observed an efficacity of tryptophan in mania, especially in the hyperactivity. SHEARD[27] has shown that a lithium inhibition of the *p*-chlorophenylalanine induced aggressivity. Lithium may act by increasing serotonin synthesis, which quite agrees with the increased turn-over of brain serotonin observed by some authors[23–25]. However, this hypothesis is not in agreement with the antagonistic effect of lithium on 5-HTP induced head-twitches in mice, observed by KISELEVA and LAPIN[28]. Thus the simultaneous administration of dexamphetamine and chlordiazepoxide may disturb a norepinephrine-serotonin balance, and it might be prevented by lithium. This effect might explain the action of lithium in mania.

Résumé. L'administration simultanée de dexamphétamine et de chlordiazepoxide provoque, chez la souris, un syndrôme d'hyperactivité, objectivé à l'aide d'une planche à trous. Ce phénomène est inhibé par l'administration préalable de lithium, mais aussi par des inhibiteurs de dopamine-β-hydroxylase et par les précurseurs de sérotonine. Ces résultats suggèrent que le lithium pourrait agir sur une balance noradrénaline sérotonine qui serait perturbée dans la manie.

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Influence of Lithium Chloride on Adrenergic Mechanisms in Ventricle of Frog Heart and Guinea-Pig Left Atrium

The catecholamine uptake system by sympathetic nerve endings of the heart is a process requiring sodium in the incubation medium[1–6]. However, lithium can replace sodium ions in the mechanism generating the cardiac action potential[7]. Splenic slices were able to accumulate H^3-norepinephrine (H^3NE) (60% of their controls) after their depletion of adrenergic transmitter by sodium deprivation in the incubation medium[8]. These facts prompted us to study if the NE uptake system by isolated frog ventricle can be modified by the replacement of sodium by lithium chloride in the incubation medium. On the other hand, the replacement of sodium by lithium in the external medium would depress the sodium-potasium pump activity as sodium concentration into the cell is reduced[9]. The facts lead us to think that the presence of lithium chloride (LiCl) in the incubation medium would prevent the NE incorporation by nerve endings.

In the present paper we have elucidated whether the prevented NE uptake by lithium is or is not produced by a cocaine-like effect by which a supersensitivity to the NE could be expected.

Methods. Experiments with frog ventricle. Ventricles (*Rana pipiens*) were prepared and mounted as previously described by FURCHGOTT et al.[10] for isolated atrium of guinea-pig. Halves were suspended in an organ bath containing 20 ml of regular Ringer solution of the following composition (expressed in m*M*): NaCl, 103.4; KCl, 1.013; $CaCl_2$, 0.9009; CO_3HNa, 1.851, containing 10^{-5} g/ml of ethylene diaminetetraacetic acid (EDTA). A mixture of 95% O_2 and 5% CO_2 was bubbled through the bathing solution. All preparations were electrically driven at a frequency of 30 beats/min. Ventricles were attached to a force-displacement transducer Grass model FT03, and mechanical activity was recorded by means of a Grass Polygraph. Each ventricle was subjected to a resting tension of 1 g. Under their respective conditions, halves were then incubated with 5 ng/ml of D,l-H^3NE for 5 min and then thoroughly washed. 4 additional washes were given over the subsequent 40 min period, at the end of which the halves were removed for analysis of radioactivity. All preparations were performed at room temperature. The catecholamine extraction was performed according to the method of ANTON and SAYRE[11] and radioactivity was counted in a Nuclear Chicago Liquid Scintillation Spectrometer model 725. All samples were corrected for quenching with an automatic external reference standard. Under our working conditions, the radioactivity present in the alumina eluates cannot be ascribed to metabolites of H^3NE but to H^3NE itself[12]. H^3NE is expressed in terms of disintegrations per min/g of tissue (dpm/g). When we refer to H^3NE uptake, we mean H^3NE uptake and retention by isolated ventricle of frog heart. Statistical significance of the difference between means was determined by the *t*-test for paired data. In the experiments with lithium chloride (LiCl), sodium chloride of the Ringer solution was replaced by equimolar amounts of LiCl.

Experiments with isolated atrium of guinea-pig. Atria were mounted in a similar manner as that described above, but suspended in Krebs bicarbonate solution. In the experimental preparations, 100 m*M*/l of LiCl was added to the normal Krebs whereas control preparations received equimolar amounts of NaCl. All preparations were performed at 37 °C. The drugs used were: D-l-norepinephrine-7-H^3-hydrochloride, specific activity 16.7 Ci/m*M* (New England Nuclear Corp.); norepinephrine

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H^3-norepinephrine uptake by isolated ventricle of the frog heart perfused with lithium chloride

N [a]	Ringer	H^3NE present during incubation	H^3NE in tissue 45 min after washout (dpm/g) [b]
6	Normal	5 ng/ml (5 min)	251.801 ± 49020
6	With LiCl	5 ng/ml (5 min)	14.291 ± 5.321

Control: normal perfusate sodium chloride concentration 103, 4 mM. Experimental: Sodium chloride replaced by equimolar lithium chloride. [a] Number of paired experiments. [b] Means ± SEM.

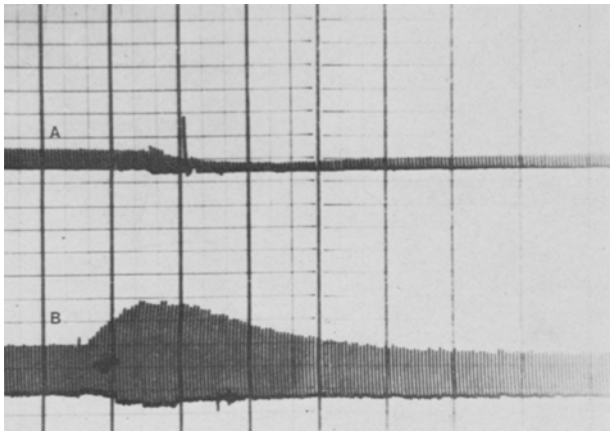

Fig. 1. Isometric contraction from stimulated left atrial preparations obtained from a reserpine-treated guinea-pig. B) records from treated half of atrium (100 mM of lithium chloride). A) records from control half of atrium (equimolar amount of sodium chloride).

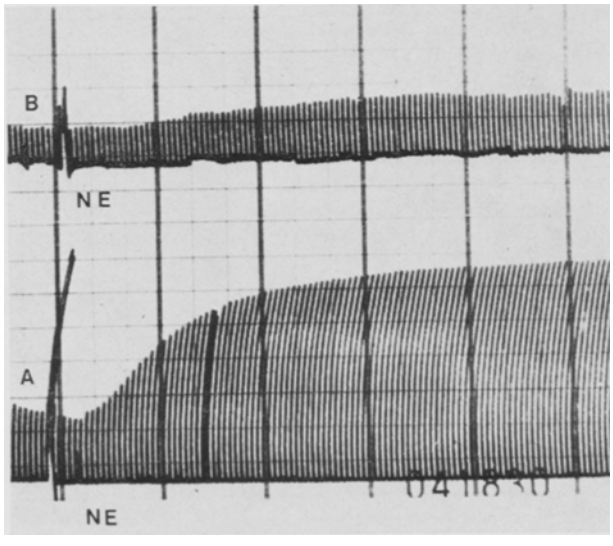

Fig. 2. Isometric contraction from stimulated left atrial preparations obtained from a reserpine-treated guinea-pig. A positive inotropic response to norepinephrine appeared only in the control preparation. B) Experimental half: Normal Krebs plus 100 mM of lithium chloride A) Control half: Normal Krebs plus equimolar amounts of sodium chloride. NE, 10^{-6} g/ml of norepinephrine.

bitartrate (Nutritional Biochem. Corp.); tyramine hydrochloride (Nutritional Biochem. Corp.); reserpine (Ciba). The doses are expressed in terms of g/ml of the bath solution.

Results. Experiments with isolated ventricle of frog. In 6 paired experiments, one half was suspended in a defficient sodium Ringer with LiCl (see methods). After a 30 min period, both halves were incubated with 5 ng/ml of H^3NE for 5 min, and were then washed: the control preparation with normal Ringer and the experimental preparation with the lithium chloride Ringer. In the Table we can observe how the experimental preparation shows a great blockade of the H^3NE uptake with respect to the control ($p < 0.01$).

Experiments with guinea-pig. In another series of experiments after a resting period of 30 min in normal Krebs, 100 mM/l of LiCl was added to the normal Krebs in the experimental preparation, whereas equimolar amounts of NaCl were added to the normal Krebs in the control preparation. In all the experiments a positive inotropic response was observed in the experimental preparation, whereas a negative inotropic response appeared in controls. In another series of experiments with reserpinized animals (5 mg/kg, 18–24 h before), after 30 min rest; tyramine (10^{-6} g/ml) was added to each preparation to test the absence of inotropic effects. After washing out the tyramine, 100 mM/l of LiCl was given to the experimental preparation and an equimolar amount of NaCl was given to the control. In Figure 1 we can observe how in both preparations no response is obtained to tyramine. Positive inotropic response to LiCl is obtained in the experimental preparation. The same negative inotropic response to NaCl described for unreserpinized animals is obtained in controls. In another series of experiments, reserpinized preparations were treated with tyramine (10^{-6} g/ml); no inotropic response was obtained. As with the preceding series, normal Krebs was replaced by hyperosmotic Krebs. Positive inotropic response was obtained in experimental preparations and negative inotropic response in control preparation. After a 30 min period, isometric contraction was restored to normal. Accumulative amounts of NE was given (10^{-6}, 3×10^{-6} and 10^{-5} g/ml). In all experiments, positive inotropic response was gradually obtained in control preparations whereas no response (or insignificant response to the greatest doses) was obtained in experimental preparations (LiCl treated). Figure 2 shows a typical experiment of this series. Finally preparations were washed in their respective Krebs and after 30 min rest, tyramine was given to both preparations. A positive inotropic response appeared only in the NaCl-treated preparation.

Discussion. The results obtained in the present paper show that LiCl failed to replace NaCl in the H^3NE uptake process by the isolated frog ventricle (Table). However, the blockade of the exogenous or endogenous NE uptake by the adrenergic nerve endings lead to a supersensitivity phenomenon produced by the great concentration of the amine at the level of the β-adrenergic receptor. The presence of lithium by blocking the incorporation, would be expected to evoke a cocaine-like effect. However, in a series of experiments with slices of isolated frog ventricle (unpublished data) the presence of lithium did not produce any potentiation of the response to NE as compared with untreated controls. In a previous work [13] we suggested that the sensitivity to NE of the

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β-adrenergic receptors of the frog heart could be modified under certain experimental or physiological conditions. In earlier reports from our laboratory [14–19], we have pointed out that the NE uptake system in cold blood animals differs in some aspects from that in hot blood species. In order to discard the possibility that this fact was dependent upon the blood temperature, we carried out a series of experiments with isolated atrium of guinea-pig. The results obtained showed that LiCl produces a positive inotropic response per se that immediately falls to a control value. The possibility that this positive inotropic response could be due to an immediate release of endogenous NE produced by lithium was discarded in a series of experiments with reserpinized animals in all of which appeared this response (Figure 1). We do not believe that the competitive antagonism potassium-lithium described by PLOEGER and DEN HERTOG [20] could be responsible for this response. The hypertonicity of the incubation medium could be a factor to take into account but in control preparations with a hypertonic Krebs (with NaCl) the inotropic positive response did not appear; on the contrary, an inotropic negative response is produced which is immediately restored to normal values (Figure 1). On the other hand, the sensitivity to NE is smaller in LiCl-treated preparations in comparison with controls treated with an excess of sodium, and maximal response to NE was not obtained in any of the LiCl-treated experiments (Figure 2).

These results support the idea that in this sense lithium has 2 different effects on the sympathetic nerve endings of the heart; 1. a neuronal action with a blockade of the catecholamine uptake (uptake 1) and 2. an extraneuronal action with a great decrease in the sensitivity of the β-adrenergic receptor. This could be the reason why the cocaine-like effect not appear. On the other hand, the treatment with tyramine after incubation with NE does not produce a positive inotropic response in lithium-treated preparations (Figure 3). This could be explained if we assume that lithium not only blocks the NE uptake but also blocks the transfer site of tyramine across the neuronal membrane. Further investigations are being carried out in our laboratory in order to reach a satisfactory conclusion.

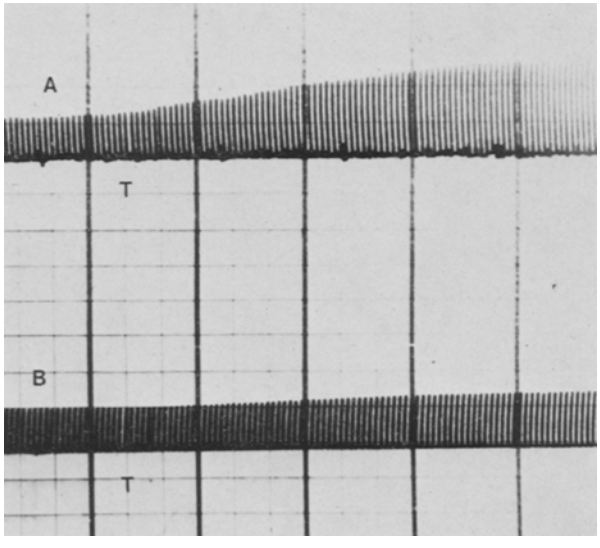

Fig. 3. Isometric contraction from stimulated left atrial preparation obtained from a reserpine-treated guinea-pig after incubation with norepinephrine. A positive inotropic response to tyramine (T) appeared only in the control preparation. B) Experimental half: normal Krebs plus 100 mM of lithium chloride. A) Control half: normal Krebs plus equimolar amounts of sodium chloride. T, 10^{-6} g/ml of tyramine.

Resumen. El cloruro de litio se comporta como bloqueante de la incorporacion de H^3NE al ventriculo aislado de *Rana*. En auricula aislada de cobayo el cloruro de litio produce efecto inotropico positivo per se y aumenta el umbral de excitabilidad a la NE administrada exogenamente.

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Types of Cell Contacts in Arterial Smooth Muscle

Some form of close contacts occurs between smooth muscle cells of arterial blood vessels. These have often been designated as nexuses or gap junctions [1, 2], though the characteristic five-layered appearance of gap junctions seen after the usual fixation and double staining procedure has been demonstrated only in larger vessels [3–7]. Since it is now recognized that several types of cell junctions may occur in various kinds of smooth muscle [8] and that gap junctions are absent from at least one kind which exhibits cell-to-cell electrical coupling [8, 9], it is of interest to examine which types of contacts may be involved in electrical coupling in arteries and terminal arterioles.

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