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Effects of Lithium and Myoinositol on the Rat Bisected Vas Deferens

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Abstract – 1. The effects of lithium (Li^+) on the concentration-response curves (CRC) to norepinephrine (NE) and acetylcholine (Ach) on the bisected rat vas deferens (RVD) were investigated, as well as its action on the neuronal uptake of [^{3}H] NE.

2. Li^+ did not affect the 50% effective concentration (EC_{50}) of NE and Ach in the epididymal (EP) portion of the RVD.

3. Li^+ caused a significant increase of the EC_{50} to NE and Ach in the prostatic (PP) portion of the RVD. This shift to the right of the CRC to NE was prevented by the presence of myoinositol.

4. Incubation of the PP of the RVD with Li^+, increased the neuronal uptake of NE. The simultaneous incubation with myoinositol prevented this increase.

5. After the pre-treatment of the rats with 6-hydroxydopamine (6-OHDA), or in the presence of cocaine, Li^+ failed to desensitize the PP of the RVD to NE.

6. These results suggest that the effect of Li^+ on the PP of the RVD occurs mainly at the pre-synaptic level and may be related to the increase of neuronal uptake and to the interference of Li^+ on phosphatidylinositol hydrolysis.

Key Words: Lithium, myoinositol, smooth muscle

INTRODUCTION

The smooth muscle contraction caused by norepinephrine (NE) is widely accepted as being due to the increase of cytosolic free Ca^{2+} concentration that could result from the opening of Ca^{2+} channels located in the plasma membrane (Bülbring and Tomita, 1987), or from the release of Ca^{2+} from internal stores (Rasmussen, 1986), or a combination of both events (Irvine, 1992). It has been proposed that these two mechanisms responsible for the increase in intracellular free Ca^{2+} concentrations are linked to pharmacologically distinguishable α_1-adrenoceptor subtypes (Han *et al.*, 1987).

We have recently reported that the α-adrenergic receptor subtypes in the rat vas deferens (RVD) mediating the contractile effect of NE have different pharmacological properties and are asymmetrically distributed along the organ (Queiroz-Neto and Ballejo, 1993). In these experiments we have shown that ryanodine, an alkaloid that interfers with Ca^{2+} release from the sarcoplasmatic reticulum (Waterhouse *et al.*, 1987) reduced the contractile response to NE only in the prostatic portion (PP) of the RVD suggesting that the α_1-adrenoceptor mediating this response are linked to the release of Ca^{2+} from intracellular stores. In addition, it is also widely accepted that the release of Ca^{2+} from intracellular organelles involves phosphatidylinositol (PI) hydrolysis and D-myo-inositol (1,4,5)-trisphosphate (IP_3) production (Berridge and Irvine, 1984).

The PI metabolism may be largely affected by Li^+. This element is known to inhibit the enzyme myoinositol 1-phosphatase (Hallcher and Sherman, 1980) and consequently to prevent the convertion of myoinositol 1-phosphate into inositol, which is the precursor for the synthesis of phosphatidylinositol. According to Berridge *et al.* (1982), continued cell stimulation in the presence of Li^+ may gradually deplete the membrane content of PI and this in turn could decrease the sensitivity of those receptors in which inositol phospholipids are important components of the transducing mechanisms.

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In addition to its effects on PI turnover, Li^+ may desensitize the adrenoceptor system by increasing the NE neuronal uptake, it has been reported in human platelets (Murphy *et al.*, 1969), brain synaptosomes (Baldessarini and Yorke, 1970), rat seminal vesicles (Patel *et al.*, 1979) and dog saphenous vein (Beaty *et al.*, 1981).

In our investigation we elected to use the RVD not only because it is considered to be an appropriate preparation to investigate the adrenergic transmission, but also because there is considerable evidence that indicate that both portions (prostatic and epididymal) of this organ differ in many pharmacological aspects, like NE neuronal uptake pattern (Avellar *et al.*, 1990) and subtypes of α_1-adrenergic receptors involved in the contraction caused by adrenergic agonists (Queiroz-Neto and Ballejo, 1993). Thus, we have investigated the action of Li^+ on the isometric contraction of the prostatic and epididymal portions of the RVD induced by NE and Ach, and verified its action on the neuronal uptake of [^{3}H] norepinephrine in the PP.

MATERIAL AND METHODS

Drugs

The drugs used were obtained from the following sources: Acetylcholine chloride, 6-Hydroxydopamine hydrochloride, Lithium acetate dihydrate, myo-inositol, L-Norepinephrine bitartrate, and Rubidium chloride, from Sigma. L-[7-^{3}H]-Norepinephrine from NEN Du-Pont, and Cocaine chloride, Ascorbic Acid and pure degree salts for physiologic saline solution, from Merck.

Tissue preparation

Vasa deferentia from sacrificed male Wistar rats (180–250 g) was dissected free of its connective tissue sheath and blood vessels in a incubation medium of the following composition (mmol.l^{-1}) NaCl 135.0, KCl 5.0, $CaCl_2$ 1.5, NaH_2PO_4 0.1, $NaHCO_3$ 15.0 and glucose 5.5, bubbled with 95% O_2 and 5% CO_2 (pH 7.4) and maintained at 32°C.

Record of vas deferens contraction

Each vas deferens was bisected into prostatic and epididymal halves. These portions were suspended under 0.5 g tension in 10 ml chambers containing the physiologic saline solution. The longitudinal contractions were recorded with an isometric transducer (BG 25 g, Kulite) and the records displayed on a Gould RS-3400 polygraph.

The preparations were allowed to equilibrate for 45 min before drug addition. Two cumulative (van Rossun, 1963) concentration-response curves (CRC) for NE or Ach were obtained for each preparation. The former was obtained immediately after the equilibration period, and the later, 60 min after the first one. During this period preparations were kept in contact with lithium (1 mmol.l^{-1}), rubidium (1 mmol.l^{-1}), and/or myo-inositol (10 mmol.l^{-1}). The incubation medium was renewed every 15 min. When cocaine was used, it was added to the incubation medium (30 μmol.l^{-1}) 10 min before the CRC were obtained. The animals chemically denervated with 6-OHDA, were treated as suggested by Westfall and Fedan (1975).

Measurement of [^{3}H]-Norepinephrine uptake

The PP of the RVD were opened longitudinally as recomended by Avellar *et al.* (1990), mounted on the tip of a plastic rod and suspended under 0.5 g tension in 10 ml of the incubation medium bubbled with O_2 (95%) and CO_2, and maintained at 32°C.

After a 60-min pre-incubation period in the presence or absence of lithium (1 mmol.l^{-1}) and/or myo-inositol (10 mmol.l^{-1}), tissues were incubated for 2 min in a medium containing 1 mCi.ml^{-1} of [^{3}H] norepinephrine (specific activity 30 Ci.mmol^{-1}) and unlabelled norepinephrine to make up a final amine concentration of 0.5 μmol.l^{-1}.

After the exposition to the radioactive material, each tissue was dipped sequentially into 5 tubes containing 10 ml of ice-cold amine-free medium for 30 sec each, dried on filter paper, weighed and transferred to scintillation vials containing 0.5 ml of NaOH (0.5 N). After standing 16 hr at 40°C we added 5 ml of scintillation fluid. About 20 hr after this procedure, radioactivity was measured in a Packard 1500 tri-carb scintillation spectrometer.

To measure the extra neuronal incorporation of NE, we conducted experiments in which we incubated the contralateral RVD PP at 0°C and substituted sucrose for sodium in the physiological solution. The difference between total and extra neuronal incorporation was taken to represent the neuronal uptake of [^{3}H] norepinephrine.

Calculations and statistics

The results were analysed by determining the changes in the sensitivity (EC_{50}) and responsiveness (maximal response) to the agonists, induced by the different treatments, and also by determining the neuronal uptake of NE in the presence or absence of lithium and/or myo-inositol.

Data were analysed statistically by the Student's *t*-test to compare two means. ANOVA followed by Tukey's test was utilized for multiple comparisons. The level of significance was set at $p < 0.05$.

RESULTS

Incubation of the PP of the RVD for 60 min with lithium (1 mmol.l^{-1}) caused a statistically significant shift to the right of the CRC to NE and Ach (Fig. 1), without affecting the maximal responses (Table 1) to these agonists. The increase of the EC_{50} of NE and Ach due to the effect of Li^+ was prevented by the concomitant incubation of the preparation with *myo*-inositol (Fig. 1 and Table 1). In the experiments where rubidium was substituted for lithium, this element failed to affect the CRCs (data not shown).

Incubation of the EP of the RVD, with Li^+, under the same conditions, did not cause any significant alteration of the CRCs to NE and Ach (data not shown).

As can be seen (Fig. 2 and Table 2), when we abolished the presynaptic component of the response of the PP to NE, by adding cocaine (30 μmol.l^{-1}) into the physiological solution, or when the rats were previously treated with 6-OHDA, the presence of lithium did not cause desensitization of the preparation. Contrarily, it can be seen in these cases, an increase in maximal response in the presence of lithium (Table 2).

After incubation of the PP of the RVD with Li^+, the neuronal uptake of [^{3}H] NE was significantly increased (Fig. 3). The presence of myo-inositol prevented this increase.

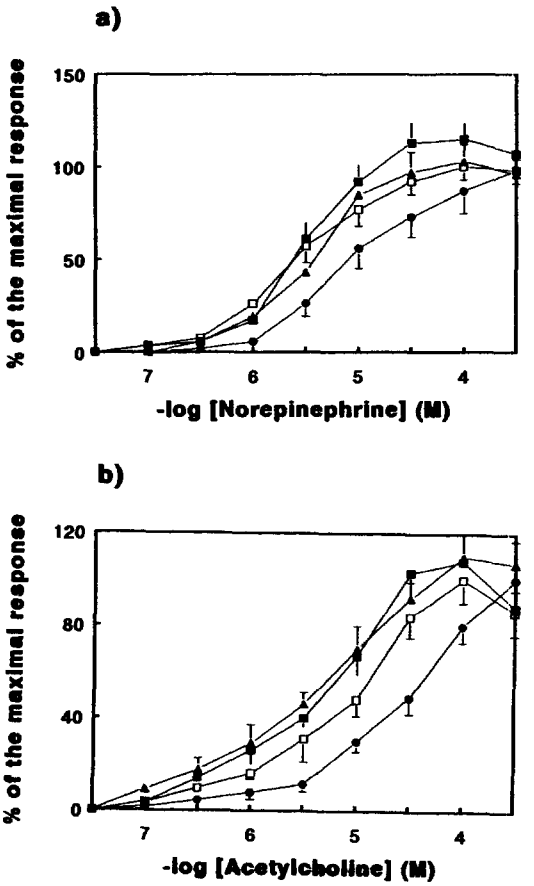

Fig. 1. Effect of lithium 1 mM (●), *myo*-inositol 10 mM (▲), lithium 1 mM + *myo*-inositol 10 mM (■) and control (□) on the CRC to norepinephrine (a) and acetylcholine (b) in the prostatic half of the RVD. Lithium and *myo*-inositol remained in contact with the preparations for 60 min before the addition of the agonists. The points are the mean ± SEM: (a) $n = 6$, (b) $n = 5$.

DISCUSSION

The results obtained in the present investigation confirm our previous findings that the α_1-adrenoceptors, which mediate the contractile response to norepinephrine in the prostatic and epididymal portions of the rat vas deferens, have different properties. Lithium, an element that has been described to exert its therapeutic action by interfering with the metabolism of phosphoinositides (Hallcher and Sherman, 1980), caused a significant desensitization of the prostatic but not of the epididymal postion of the organ. This finding is entirely consistent with our previous report (Queiroz-Neto and Ballejo, 1993), which showed that ryanodine reduced the contractile response to NE only in the PP

Table 1. Changes induced by incubation (60 min) of the prostatic portion of the rat vas deferens with lithium (1 mM) and/or *myo*-inositol 10 mM (MI), on the effective median concentration (EC_{50}) and maximal response (MR) to norepinephrine and acetylcholine

	Group	N	Mean MR ± SEM (g)	Agonist (95% CI) EC_{50} (M)
NE	Control	12	0.67 ± 0.06*	2.5 (1.91-3.41).10^{-6}*
	Li^+	06	0.67 ± 0.11*	9.4 (4.67-18.9).10^{-6}†
	MI	06	0.69 ± 0.07*	3.4 (1.90-4.68).10^{-6}*
	MI + Li^+	06	0.79 ± 0.10*	3.1 (1.76-5.41).10^{-6}*
Ach	Control	11	0.63 ± 0.09*	9.7 (5.92-16.1).10^{-6}*
	Li^+	05	0.66 ± 0.06*	33.0 (23.1-47.4).10^{-6}†
	MI	05	0.68 ± 0.01*	5.3 (2.12-13.1).10^{-6}*
	MI + Li^+	05	0.68 ± 0.02*	7.2 (4.45-11.7).10^{-6}*

Results are reported as mean ± SEM. *N*, number of experiments. Means followed by different symbol are statistically different ($P < 0.05$, Tukey test).

Table 2. Changes induced by incubation of the prostatic portion of the rat vas deferens with lithium (1 mM) for 60 min on the effective median concentration (EC_{50}) and maximal response (MR) to norepinephrine, in the presence or absence of cocaine (30 mM), or in rats previously treated with 6-hydroxydopamine

	Group	*N*	Mean MR ± SEM (g)	Agonist (95% CI) EC_{50} (M)
NE	Control	12	0.67 ± 0.06*	2.5 (1.91-3.41).10^{-6}*
	Li^+	06	0.65 ± 0.06*	13.8 (4.84-21.0).10^{-6}*
	Cocaine	06	0.98 ± 0.16*†	1.3 (0.73-2.66).10^{-6}*
	Cocaine + Li^+	06	1.35 ± 0.31†	2.9 (0.90-9.84).10^{-6}*
	6-OHDA	06	1.02 ± 0.15†	0.5 (0.31-0.84).10^{-6}*
	6-OHDA + Li^+	06	1.24 ± 0.22†	0.3 (0.24-0.44).10^{-6}*

Results are reported as mean ± SEM. *n* = number of experiments. Means followed by different symbol are statistically different ($P < 0.05$, Tukey test).

of the RVD suggesting that the α_1 adrenoceptors mediating this response are linked to the release of calcium from intracellular stores. Another striking evidence of the participation of phosphoinositide metabolites in the contraction of the PP but not of the EP of the RVD to NE is that the desensitization of the PP to this agonist due the action of Li^+ was prevented by supplying the medium with myo-inositol.

Furthermore, participation of a presynaptic component was characterized by the results that show that in the presence of cocaine, or when the rats were pharmacologicaly denervated with 6-OHDA, Li^+ failed to increase the EC_{50} of NE in the PP of the RVD. It was also found that the presence of Li^+ increased the neuronal [^{3}H] NE uptake in the PP of the RVD. This result agrees with those of Avellar *et al.* (1990) who found an age-related loss of function in the neuronal uptake of NE in the prostatic but not in the epididymal portion, and with those of Markus and Avellar (1992) who confirmed the potentiating effect of lithium on neuronal [^{3}H] NE uptake in the PP but not in the EP. In addition, the fact that this increase of neuronal [^{3}H] norepinephrine uptake due the action of Li^+ was blocked by concomitant incubation of the tissue with myo-

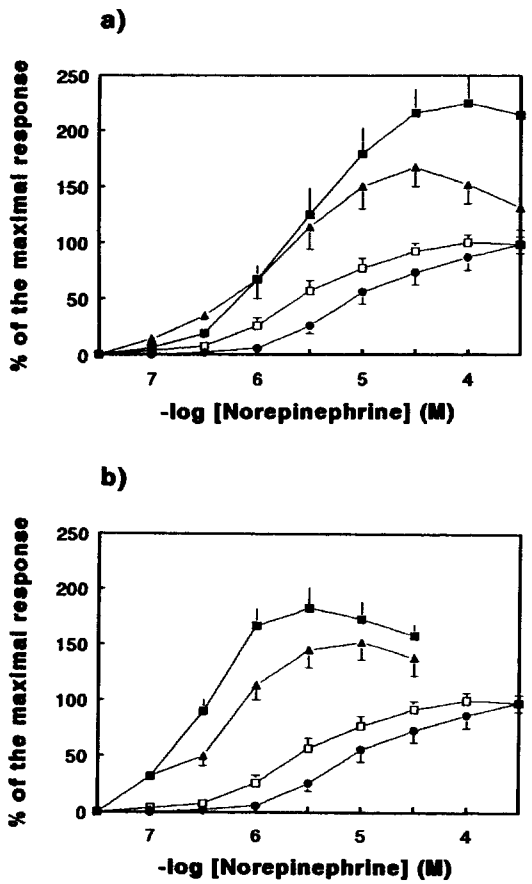

Fig. 2. Effect of various treatments on the CRC to NE in the prostatic half of the RVD. (a) control (□), lithium 1 mM (●), cocaine 30 fM (▲), and cocaine 30 mM + lithium 1 mM (■). (b) control (□), lithium 1 mM (●), animals pretreated with 6-OHDA (▲) and animals pretreated with 6-OHDA + lithium 1 mM (■). Lithium remained in contact with the preparation for 60 min before the addition of the agonist. The points are the mean ± SEM of 6 experiments.

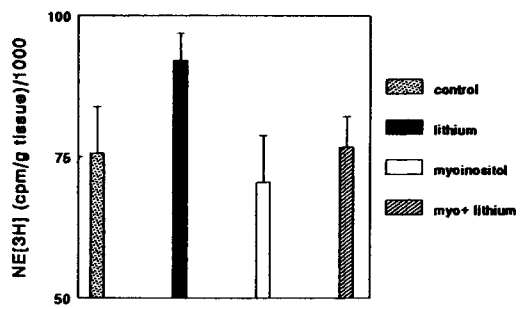

Fig. 3. Neuronal uptake of [^{3}H] norepinephrine in the prostatic half of the RVD. Control, lithium 1 mM, *myo*-inositol 10 mM and lithium 1 mM + *myo*-inositol 10 mM. Tissues were incubated for 60 min in the presence or absence of lithium and/or *myo*-inositol and then incubated for 2 min in a medium containing 1 mCi.ml^{-1} of [^{3}H] NE and unlabeled NE to make up a final concentration of 0.5 mM. Results are expressed as CPM (counts per min) of each experimental group, corrected by the weight of each organ. Values are given as mean ± SEM from 11 experiments each. *$P < 0.05$ compared with control group.

inositol suggests an involvement of phosphatidylinositol metabolites in the neuronal uptake process.

With regard to the effect of lithium on the CRC to Ach, it is known that this agonist causes contraction of the PP of the RVD through stimulation of nicotinic receptors (Kasuya and Suzuki, 1978). According to Jayasundar and Vohra (1977) and Carneiro and Markus (1990), this stimulation could induce NE release from adrenergic endings. Thus, we may suggest that the effect of lithium on the CRC to Ach is due to its action on the adrenergic transmission, and not on the cholinergic one itself. In conclusion, although further studies will be necessary to clarify the mechanism by which lithium interferes with the action of norepinephrine, the present observations provide evidence that the effect of lithium on the prostatic portion of the rat vas deferens occurs mainly at the presynaptic level and may be related to the increase of the neuronal uptake of norepinephrine and to the interference of this element on the hydrolysis of phosphatidylinositol.

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