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The effects of acute and chronic lithium treatment on rat submandibular salivation

J Popović[1], E Kršljak[2], L Grbović[3], D Stojić[1]

Departments of [1]Pharmacology and [2]Physiology, Faculty of Stomatology; [3]Department of Clinical Pharmacology, Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia & Montenegro

OBJECTIVE: Acute and chronic actions of lithium on salivation induced by agonists associated with receptor-linked hydrolysis of membrane inositol phospholipids (carbachol and phenylephrine) and by agonist linked to activation of adenylate cyclase (isoproterenol) were investigated.

MATERIAL AND METHODS: In anaesthetized rats, submandibular salivation induced by intravenous injection of carbachol, phenylephrine and isoproterenol, was measured and expressed as volume of fluid (μl) elicited per 100 mg wet weight of each gland per minute. The experiments were repeated after acute and chronic treatment of lithium (7 mg kg^{-1}). The results were analysed with unpaired t-test.

RESULTS: Chronic, but not acute lithium treatment significantly decreases carbachol- and phenylephrine-induced salivation while isoproterenol-induced salivation was not changed neither after acute nor after chronic administration of lithium.

CONCLUSION: The results suggest that hyposalivation during chronic lithium therapy could be mediated by alterations in the phosphatidylinositol cycle and a consequent lack of inositol after agonist stimulation.
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Keywords: lithium; salivation; acute and chronic treatment

Introduction

The obvious importance of saliva in maintaining the integrity of the oral environment is well known. Large decreases in salivary gland function, regardless of the aetiology, may have drastic consequences for oral health (Rundegren *et al*, 1985; Bowen *et al*, 1988). Many drugs have hyposalivation as adverse effect which leads to subjective discomfort and may be associated with dental caries and gingivitis. It is well established that a variety of psychoactive drugs have profound effects on salivary gland function and cause xerostomia mainly by modifying mechanisms of secretion coupled to muscarinic cholinergic receptor activation. Psychoactive drugs that are commonly accompanied by xerostomia are: tricyclic antidepressants, antipsychotics of phenotiazine class and lithium (Mörnstad *et al*, 1986; Markitziu *et al*, 1988; Szabadi and Tavernor, 1999). While the antisialogouge effect of tricyclic antidepressants and phenothiazines is mediated via blockade of muscarinic receptor (Mörnstad *et al*, 1986), the precise mechanism of an antisialogouge effect of lithium remains unclear.

Concerning chronic lithium's clinical antimanic and antidepressant action, a number of studies have indicated that lithium appears to act upon postreceptor component of signal transduction pathways. This mechanism includes inhibition of inositolmonophosphatase in phosphatidylinositol cycle by interfering with neurotransmiter-induced polyphosphatidylinositol hydrolysis, in both peripheral tissues and brain (Berridge *et al*, 1982; Nahorski *et al*, 1991; Phiel and Klein, 2001). There is, also, evidence that some of the chronic effects of lithium on brain function may be mediated by alterations in adenylate cyclase activity (Newman and Belmaker, 1987; Colin *et al*, 1991).

The present study investigated the effect of acute and chronic lithium treatment on rat submandibular salivation because of muscarinic cholinergic and α_1 adrenergic receptor stimulation, which trigger cycle of phosphatidylinositol turnover, and on the contrary, the effect of lithium on salivation induced by β adrenoceptor activation coupled to activation of adenylate cyclase.

Method

The study was performed on adult rats (165) of Wistar strain of either sex, weighing 280–330 g, anaesthetized with urethane. Rats had access to rat chow and water *ad libitum* but were fasted for 24 h prior to

Correspondence: J Popović, Dr. Subotića 1, 11000 Belgrade, Serbia & Montenegro. Tel.: +38111682373; Fax: +38111685361; E-mail: majajel2002@yahoo.com
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experimentation. The submandibular glands were exposed and separated from the adherent sublingual glands, the ducts of which were ligated. The ducts of both parotid glands were ligated also. The submandibular saliva secreted after administration of different sialogogic agents was collected from the floor of the mouth with capillarymicropippete (Iwabuchi and Masuhara, 1995) during 5 min time; 10 additional minutes were allowed until the next dose was injected.

Dose–response curves were obtained by sequential intravenous injection of increasing doses of carbachol (5–25 μg kg^{-1}), phenylephrine (0.5–1.5 mg kg^{-1}) and isoproterenol (50–175 μg kg^{-1}). One animal received only the doses for dose response curve for one agonist. The doses of agonists were chosen according to preliminary experiments which obtained the results of the significant rate of salivation. Secretogouge effect of each of these drugs was measured one hour after acute oral administration of lithium as well as after chronic (7 days) oral administration of lithium. A dose of lithium used in all experiments was 7 mg kg^{-1}, a dose that correlate with serum lithium level of 1.2 mM, which is in the therapeutic range. Carbonate salt of lithium dissolved in distilled water was used. At the end of each experiment, submandibular glands were carefully removed and weighed (262 ± 5 mg in controls; 258 ± 6 mg in rats after acute treatment of lithium; 260 ± 5 mg in rats after oral chronic treatment of lithium). Flow rates were calculated from the volume of fluid (μl) elicited per 100 mg wet weight of each gland per minute (μl 100 mg^{-1} min^{-1}).

Experimental values were expressed as mean ± s.e.m. of at least five experiments and statistical comparisons were made by means of an unpaired Student's *t*-test. Animal use protocol was approved by the Ethical Committee of the Faculty of Stomatology, University of Belgrade.

All chemicals were purchased from Sigma Chemical (St Louis, MO, USA), except lithium carbonate which was obtained from Srbolek (Belgrade, Serbia & Montenegro).

Results

Intravenous injection of carbachol, a muscarinic agonist, in increasing doses of 5–25 μg kg^{-1} elicited a dose dependent flow rate of saliva from the submandibular gland with maximum response of 3,4 ± 0,2 μl min^{-1} per 100 mg wet weight (Figure 1). After acute oral administration of lithium dose–response curve for carbachol was not significantly changed (Figure 1). In contrast to that, after chronic administration of lithium, dose–response curve for carbachol showed downward-shift with reduction of maximal effect for 33%.

Phenylephrine, an α adrenergic agonist, was applied in the doses of 0.5–1.5 mg kg^{-1} and produced dose dependent increase in salivation. The dose response curve for phenylephrine was not significantly changed after acute treatment of lithium, while after chronic treatment of lithium, there was a reduction of maximal effect for 50% (Figure 2).

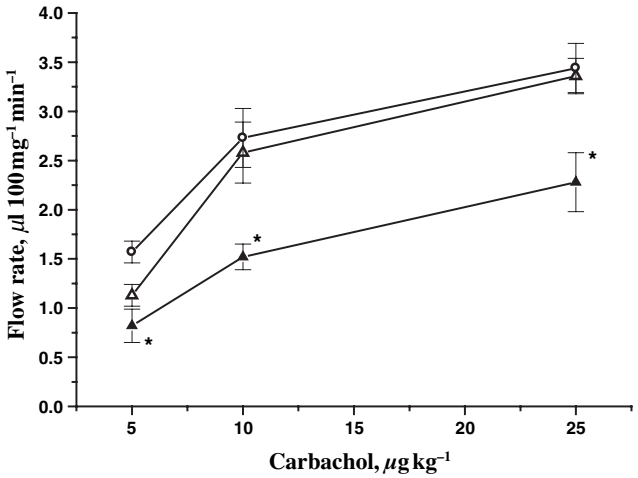

Figure 1 Dose–response curves for carbachol before (○) and after acute (△) and chronic (▲) lithium treatment in rats. Each point represents the mean ± s.e.m. of five experiments. Responses are expressed as the volume of saliva (μl) elicited per 100 mg wet weight of each gland per minute (μl 100 mg^{-1} min^{-1}). *$P < 0.05$ with respect to control

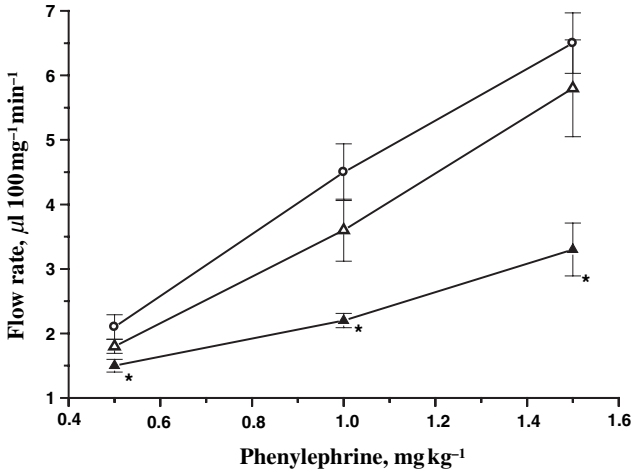

Figure 2 Dose–response curves for phenylephrine before (○) and after acute (△) and chronic (▲) lithium treatment in rats. Each point represents the mean ± s.e.m. of five experiments. Responses are expressed as the volume of saliva (μl) elicited per 100 mg wet weight of each gland per minute (μl 100 mg^{-1} min^{-1}). *$P < 0.05$ with respect to control

There was no significant change in the salivary response to isoproterenol, a β adrenergic agonist neither after acute nor after chronic administration of lithium (Figure 3).

Discussion

Inspite of the fact that activation of muscarinic receptors is by far the most potent natural stimulus for salivation, the results of our study show that salivary effect of phenylephrine, an α adrenergic agonist, is higher than the salivary effect obtained with carbachol, a muscarinic agonist. An explanation of this could be found in the fact that intravenous application of the

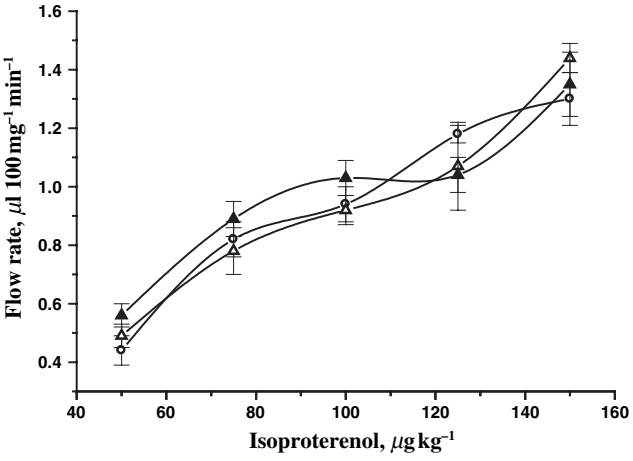

Figure 3 Dose–response curves for isoproterenol before (○) and after acute (△) and chronic (▲) lithium treatment in rats. Each point represents the mean ± s.e.m. of five experiments. Responses are expressed as the volume of saliva (μl) elicited per 100 mg wet weight of each gland per minute (μl 100 mg^{-1} min^{-1}). *$P < 0.05$ with respect to control

doses higher of the maximal dose of carbachol (25 μg kg^{-1}), used in our experiments, is followed by lethal outcome; at the same time, administration of phenylephrine at higher dose did not lead to fatalities. This problem cannot be seen after intraperitoneal application of carbachol to rats, even in dose of 100 μg kg^{-1} (Baba *et al* 1994). We also observed that, following sequential doses of phenylephrine, effect was consistent, as in the case of other α agonists, such as norepinephrine and methoxamine (Elverdin *et al*, 1984), inspite that variable vasoconstriction can occur leading to variable secretory rates. The present results are, also, in accordance with findings of Kaniucki *et al* (1984) who, in the same experimental procedure, by obtaining dose response curves for muscarinic and α adrenergic agonist applied in sequential doses, observed lower salivary effect after intravenous application of metacholine, a muscarinic agonist, and noradrenaline, an α agonist.

One of the main findings in the present study is significant reduction in salivation induced by carbachol after chronic (7 days of oral application) treatment of lithium in rats. As acute oral administration of lithium had no effect on salivary response to carbachol, it seems unlikely that the observed antisialogouge effect of lithium is a consequence of direct blockade of muscarinic cholinergic receptor. In this respect, lithium differs from many other drugs whose antisialogouge effect is underlied by their direct anticholinergic action (Scully, 2003).

Signal transduction mechanism underlying stimulation of muscarinic cholinergic receptors in salivary glands involves G-proteins activation resulting in hydrolysis of phosphatidylinositol biphosphate by phospholipase C and thereby production of phosphatidylinositol 1,4,5-triphosphate (IP_3) which leads to a rise in the intracellular calcium concentration and consequently to fluid secretion (Fleming *et al*, 1987; Nauntofte, 1992). It has been demonstrated that lithium interferes with phosphatidylinositol turnover in rat brain slices and parotid fragments, by inhibiting inositolmonophosphatase and consequently decreasing level of IP_3 in uncompetitive and time-dependent manner (Berridge *et al*, 1982). It could be suggested that the same mechanism underlies antisialogouge effect of lithium as lithium reduced cholinergically-induced salivation in uncompetitive manner; this effect was related to chronic treatment of lithium. Downes and Stone (1986), using rat parotid slices and isolated acinar cells labelled with 32 Pi, reported that lithium alone had little effect upon 32 Pi incorporation but, in combination with carbachol, it greatly reduced the phosphatidylinositol labelling response to the agonist. Furthermore, Tritsarisa *et al* (2001), have shown a reduction in cholinergically-induced production of IP_3 as well as decreased release of intracellular calcium in lithium-treated rat parotid acini. The authors concluded that this diminished production of IP_3 resulting in reduced calcium mobilization might contribute to the hyposalivation observed in patients on chronic lithium treatment.

We have also found that chronic administration of lithium reduces phenylephrine-induced secretory response in uncompetitive manner, as in the case of carbachol. Phenylephrine, as a selective α_1 agonist, produces salivation because of activation of α_1 adrenoceptor associated with phosphoinositide signalling (Fleming *et al*, 1987). The inhibitory effect of lithium on phenylephrine-induced secretion supports the suggestion that its antisialogouge effect could be a consequence of its interference with phosphatidylinositol turnover.

Several lines of evidence suggest that the action of chronic lithium treatment in bipolar disorder may not be only manifested in receptor-mediated phosphatidylinositol turnover. A variety of effects of lithium on adenylate cyclase activity in brain is reported (Newman and Belmaker, 1987; Colin *et al*, 1991). Chronic lithium treatment (4 weeks) increases levels of adenylate cyclase mRNA and protein in rat cerebral cortex (Colin *et al*, 1991). In our experiments chronic lithium treatment did not change isoproterenol-induced salivation, the effect mediated by activation of adenylate cyclase and cAMP accumulation. Our chronic treatment lasted for 7 days, a period in which Colin *et al* (1991), did not observe any effect on the levels of adenylate cyclase mRNA and protein in rat cerebral cortex.

It is of interest that in contrast to our findings, acute intraperitoneal (10 mg kg^{-1}) and chronic oral administration (1200 mg l^{-1}) of lithium chloride in rats did not significantly reduce cholinergically-induced salivation (Dehpour *et al*, 1995). Such dichotomous effect of lithium could be accounted for by the different lithium regimes used in studies. Moreover, Markitziu *et al* (1988), in a clinical study on manic-depressive outpatients on chronic lithium treatment, reported that majority of patients experienced hyposalivation, but, however, there were some that exhibited normal salivation. The authors suggested that these differences might be the result of the wide range of dosages in lithium

therapy in the studied population (Markitziu *et al*, 1988).

Beside the molecular mechanisms underlying hyposalivation during chronic lithium therapy, additional factors might be involved in salivary dysfunction. A possible association between hypothyroidism, an established side-effect of lithium therapy (Emerson *et al*, 1973; Amdisen and Andersen, 1982), and xerostomia in manic-depressive patients is likely (Markitziu *et al*, 1988). Fatty degeneration of the salivary gland parenchyma demonstrated histologically in the patients with prolonged periods of hypothyroidism (Markitziu *et al* 1993) may contribute to reduced salivary gland function. The other possibility is that reduced salivary flow is caused by general dehydration of the patient's body by lithium-induced polyuria (Gitlin, 1999).

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