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EFFECTS OF LITHIUM AND DESIPRAMINE ADMINISTRATION ON AGONIST-STIMULATED INOSITOL PHOSPHATE ACCUMULATION IN RAT CEREBRAL CORTEX

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Abstract—The effects of acute lithium and chronic desipramine administration on the inositol phosphate responses of rat cerebral cortical slices to noradrenaline, carbachol and 5-hydroxytryptamine, were determined. Acute injection of lithium (4 meq/kg s.c. 24 hr before sacrifice) significantly increased the inositol-1-phosphate response to noradrenaline, while chronic administration of desipramine (10 mg/kg i.p. for 3 weeks) produced a smaller increase in this parameter. Chronic desipramine also decreased the serotonin responses in terms of all three inositol phosphates measured. No potentiative or additive interactions between the lithium and desipramine effects were observed.

Hydrolysis of the membrane phospholipid phosphatidyl-4,5-bisphosphate (PIP_2) results in the formation of the two second messenger substances inositol trisphosphate (IP_3) and diacylglycerol, and is now accepted as a widespread mechanism for hormone- or neurotransmitter-dependent signal transduction [1–4]. In brain, this mechanism may represent a target for the action of antidepressant and psychotropic drugs. Lithium (Li) was first shown by Allison *et al.* [5] to affect this system by inhibiting the enzyme myo-inositol-1-phosphatase, thus leading to a large increase in tissue inositol-1-phosphate (IP_1) levels. This observation was used by Berridge *et al.* [6] develop an *in vitro* assay for agonist-dependent PIP_2 breakdown by measuring conversion of inositol to IP_1 in the presence of Li. The relationship of this effect to the therapeutic action of Li is, however, still unclear. Berridge *et al.* [6] predicted that chronic treatment with Li would lead to a reduction in intracellular inositol and hence depletion of the membrane content of PIP_2. Sherman *et al.* [7] found highly significant increases in IP_1 levels and decreases in inositol levels in rat cortex after both acute and chronic Li administration *in vivo*. However, these authors failed to observe the expected changes in the levels of PIP_2 or the other brain phospholipids, which could account for an Li-mediated alteration of signal transduction. In the present work we have determined the effect of acutely administered Li on agonist-dependent PIP_2 turnover, in an attempt to obtain evidence for the mechanism proposed by Berridge *et al.* [6] from *in vivo* experiments.

Several lines of evidence also point to the PIP_2 hydrolysis–IP_3 formation system as a potential target for tricyclic antidepressant action. Tricyclic antidepressants have been shown to down-regulate cortical $5HT_2$ receptors [8, 9] and to up-regulate cortical alpha-1 adrenoceptors, as measured using the specific ligand ^{3}H-prazosin [10]. Both these receptors are coupled to the PIP_2 hydrolysis system in rat cerebral cortex [11–13]. Kendall and Nahorski [14] demonstrated a reduction in 5-HT-mediated inositol phosphate production in rat cerebral cortex slices after chronic treatment with imipramine or iprindole, although no effects on alpha-1 agonist or carbachol stimulation were observed. In recent work from our laboratory [15] we have observed that chronic electroconvulsive shock (ECS) administration to rats results in an increase in alpha-1 receptor-mediated IP_1 formation in rat cortex, parallel to the increase in alpha-1 adrenoceptor number induced by this treatment [16]. No changes in carbachol- or 5-HT-mediated stimulation of the response were, however, detected in cerebral cortex.

Addition of short-term Li to chronic tricyclic antidepressant administration has been found effective in the treatment of resistant depressions by several authors [17–19]. The basis for this rapid therapeutic effect is unknown but has been suggested to be mediated via Li potentiation of 5-HT release [20] or another interaction between Li and 5-HT function. In the present work the effects of acute Li, chronic desipramine (DMI) and a combination of these agents on the inositol phosphate responses to the three agonists noradrenaline (acting via alpha-1 adrenoceptors), carbachol and 5-HT were determined in order to investigate a possible molecular basis for this interaction.

MATERIALS AND METHODS

Male albino rats (Charles River strain) were used in all experiments. The rats were housed in a temperature-controlled environment (24°) with a regular 12 hr light/dark cycle. Food and water were freely available.

The rats were divided into groups of four, each group being housed separately. Two rats in each group received i.p. injections of desipramine hydrochloride (10 mg/kg) once daily for 3 weeks, the other

two receiving saline. On the last treatment day one of the saline-injected rats and one of the DMI-treated rats received s.c. injections of LiCl (4 meq/kg). The rats were killed 24 hr after these injections, and carotid blood and residual brain tissue taken from the Li-treated animals for determination of Li levels by flame photometry.

Brain slices were prepared by cross-chopping using a McIlwain tissue chopper set at 0.35 mm. The slices were pre-incubated in Krebs–Ringer bicarbonate buffer containing 1.29 mM $CaCl_2$ and 10 mM glucose with constant shaking and gassing with 95% O_2/5% CO_2, for 60 min at 37°. Aliquots (25 μl) of packed slices (≈0.5 mg protein) were then dispersed among polypropylene tubes containing 0.45 ml Krebs–Ringer and 0.3 μM ^{3}H-inositol. The tubes were gassed for 10 min, capped, and incubation continued for a further 50 min. At the end of this period agonists (noradrenaline, carbachol, serotonin) were added in a volume of 50 μl of Krebs–Ringer bicarbonate buffer containing in addition 10 mM LiCl, so that the final LiCl concentration in the incubation was 1 mM, and the incubation continued for a further 60 min. In the case of serotonin, all incubations were performed in the additional presence of 10 μM pargyline [12]. The tubes were then centrifuged, the medium decanted and the slices washed twice with 1 ml ice-cold Krebs–Ringer. The slices were then homogenised in 1 ml of the same buffer, as described by Schoepp *et al.* [13], centrifuged, and the supernatants applied to columns containing 1 ml Dowex-1-X8 resin (formate form).

The inositol phosphates were eluted sequentially from the Dowex-1-X8 columns by a modification of the method of Berridge *et al.* [21]. IP_1 was eluted with 4 ml of 0.2 M ammonium formate/0.1 M formic acid directly into scintillation vials for counting. After this another 4 ml of the same solution was run through the columns to remove any residual IP_1, and inositol bisphosphate (IP_2) eluted with 4 ml 0.4 M ammonium formate/0.1 M formic acid. A further 4 ml of this solution was then run through the columns before collection of an IP_3 fraction with 4 ml 1 M ammonium formate/0.1 M formic acid. This fraction has recently [22] been shown to comprise a mixture of the isomers inositol-1,4,5-trisphosphate, inositol-1,3,4-trisphosphate and inositol-1,3,4,5-tetrakisphosphate, and is here abbreviated to IP_3. The recovery of ^{14}C-labelled IP_1 in the first fraction was 85–90% when this compound was applied alone, and 81–82% when it was added to slice homogenates.

RESULTS

Plasma lithium levels in the Li-treated rats were 1.04 ± 0.14 meq/l, and the corresponding brain levels 2.05 ± 0.26 meq/l (mean ± SEM of nine animals). For the animals which received both acute Li and chronic DMI, plasma Li levels were 0.98 ± 0.13 meq/l, and corresponding brain levels 1.76 ± 0.15 meq/l (mean ± SEM of nine animals). In another series of experiments, Li levels in slices from Li-treated animals dropped from 0.88 ± 0.12 meq/l to 0.18 ± 0.02 meq/l (mean ± SEM of results from three animals) after 60 min preincubation. There were no differences in basal levels of any of the inositol phosphates in incubated slices derived from animals between the various treatment groups.

Acute Li administration significantly increased the degree of stimulation of IP_1 formation by noradrenaline (Fig. 1). The degree of stimulation of IP_1 formation by noradrenaline was also increased in the DMI-treated animals (Fig. 1), but this effect was only of borderline significance ($P = 0.065$). The increase in this parameter observed in the animals which received both acute Li and chronic DMI was no greater than in those which received either treatment alone. There was no effect of any of the treatments on the degree of carbachol stimulation of the response. 5HT-induced IP_1 accumulation was

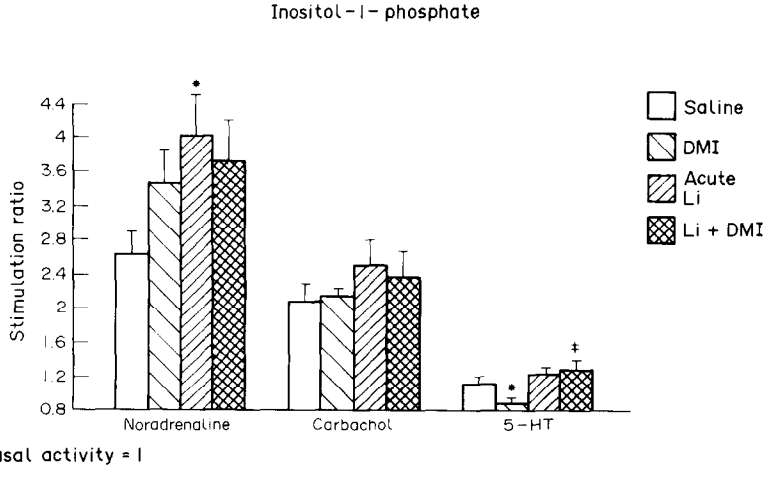

Fig. 1. The effect of acute lithium and chronic DMI treatment on accumulation of inositol-1-phosphate in rat cerebral cortical slices. Concentrations of activating agents used were: noradrenaline, 0.2 mM; carbachol, 20 mM; serotonin, 2 mM in the additional presence of 10 μM pargyline. Each bar represents the mean ± SEM of duplicate observations from nine animals in each group. * Significantly different ($P < 0.05$) from saline-treated animals by Student's *t*-test; ‡ significantly different ($P < 0.05$) from DMI-tested animals by Student's *t*-test.

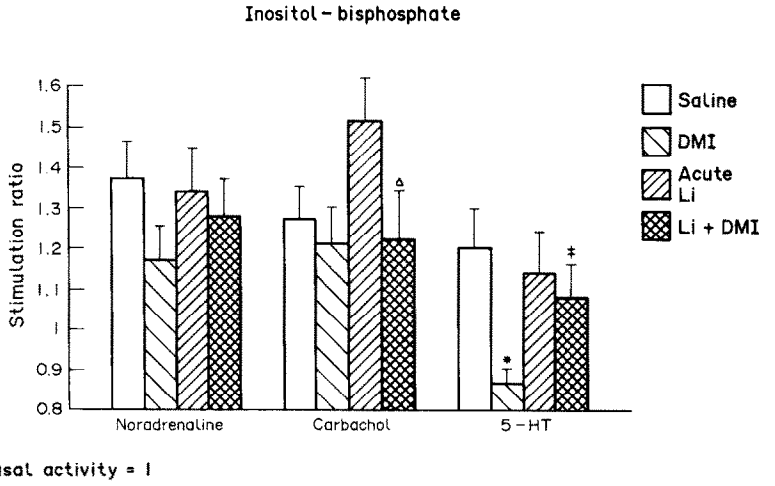

Fig. 2. The effect of acute lithium and chronic DMI treatment on accumulation of IP_2 in rat cerebral cortical slices. Experimental conditions were as in Fig. 1. * Significantly different ($P < 0.05$) from saline-treated animals by Student's t-test; ‡ significantly different ($P < 0.05$) from DMI-treated animals by Student's t-test. △, significantly different ($P < 0.05$) from Li-treated animals by Student's t-test.

significantly reduced in DMI-treated compared to control rats, to the extent that no 5-HT stimulation was observed. In rats which received both acute Li and chronic DMI, the 5-HT response was significantly increased compared to that in rats receiving DMI alone.

No effect of any of the treatments on the IP_2 response to noradrenaline were observed (Fig. 2). In the case of carbachol stimulation, rats receiving Li and DMI showed a reduced response compared to those receiving acute Li only. For 5-HT stimulation the results were exactly parallel to those obtained for IP_1, namely a reduced response in the DMI-treated rats and an increased response in rats which received both acute Li and DMI when compared to those which received DMI alone.

The IP_3 response to noradrenaline was also not affected by any of the treatments (Fig. 3). The response to carbachol was significantly reduced in the DMI treated rats, while the response in rats which received both acute Li and DMI was increased compared to those which received DMI alone. The pattern of changes in the IP_3 response to 5-HT was identical to that observed for the IP_1 and IP_2 responses to this agonist.

DISCUSSION

The present results confirm the observations of Kendall and Nahorski [14] of a decrease in 5-HT-stimulated inositol phosphate formation in cerebral cortical slices from rats treated chronically with a tricyclic antidepressant. Our incubations were performed in the presence of 1 mM Li only and inositol

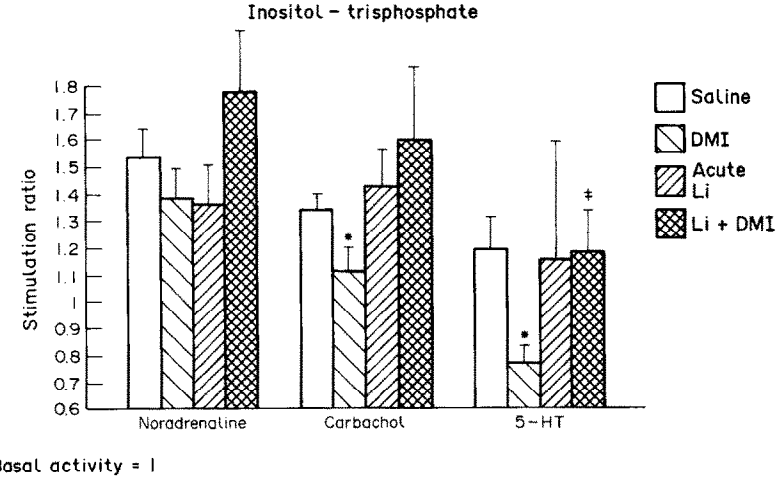

Fig. 3. The effect of acute lithium and chronic DMI treatment on accumulation of IP_3 in rat cerebral cortical slices. Experimental conditions and symbols as in Fig. 2.

phosphates were measured individually, while in the work of Kendall and Nahorski [14], 5 mM Li was included in the assay medium and a single inositol phosphate fraction was collected. The similar result obtained with these differing experimental conditions strengthens the observation, which parallels the decrease in 5-HT_2 receptor number observed after chronic antidepressant treatment [9, 23]. This observation contrasts with the lack of effect of chronic ECS on 5-HT-stimulated inositol phosphate formation [15], despite the increase in 5-HT_2 receptor number reported by several authors after this treatment [8, 9, 24].

Kendall and Nahorski [14] reported no effect of chronic DMI treatment on noradrenaline stimulation of total inositol phosphate accumulation in rat cerebral cortical slices. Our findings of a small, non-significant increase in IP_1 on noradrenaline stimulation provide a functional counterpart for the observation of Vetulani *et al.* [10] of an increase in alpha-1 adrenoceptor number after chronic antidepressant treatment, which in this case match similar findings in both parameters after chronic ECS [15, 16]. It is possible that when the inositol phosphates are combined, the increase in IP_1 accumulation due to DMI treatment will be obscured by its lack of effect on noradrenaline-induced IP_2 and IP_3 accumulation, as observed in the present study. The lack of effect of chronic DMI on the response to carbachol matches the observations of Peroutka and Snyder [23], who observed no effect of tricyclic antidepressants on muscarinic cholinergic receptor number in rat cerebral cortex.

The present work shows that acute Li injection 24 hr before the experiment results in potentiation of noradrenaline-induced stimulation of IP_1 formation, although the other parameters measured were unchanged after this treatment. This result indicates, at least for the noradrenaline response, that the effect of Li *in vitro* initially observed by Berridge *et al.* [6], and subsequently replicated by several authors [11–16], is also obtained when Li is administered *in vivo*. The residual Li concentration of 0.2 mM remaining in the slices after pre-incubation thus appeared to be effective in inhibition of inositol-1-phosphatase when combined with the 1 mM added *in vitro*. This finding is in keeping with half-maximal values of 0.5–0.7 mM for Li inhibition of inositol-1 phosphatase reported by Berridge *et al.* [6] and Batty and Nahorski [25]. Our results contrast with those of Kendall and Nahorski [26], who administered 6.75 meq/kg LiCl i.p. to rats 16–18 hr before sacrifice and obtained reductions in the responses to carbachol, elevated K^+ and 5-HT. Their experiments were, however, carried out in the additional presence of 5 mM Li *in vitro*, and it would appear that at these Li levels effects other than inhibition of inositol-1-phosphatase become evident. Further evidence for this is provided by the observation of Kendall and Nahorski [26] that the Li-induced decrease in IP formation was potentiated by pre-incubation of the slices with unlabelled inositol, in order to overcome the increase in specific activity of ^{3}H-inositol caused by Li inhibition of inositol-1-phosphatase. We have no explanation as to why in our experiments only the noradrenaline effect was potentiated by Li *in vivo*. However, the results of Kendall and Nahorski [26] may provide a clue in that they found the noradrenaline effect not to be reduced by Li under their experimental conditions, indicating differential regulation of this response.

These experiments did not show any indication of a potentiative or additive interaction between Li and DMI parallel to clinical findings in which Li addition was found to rapidly potentiate the therapeutic action of ongoing tricyclic antidepressant treatment [17–19]. Indeed the results for 5-HT stimulation of the inositol phosphate response showed that the two treatments had effects in opposite directions, and that acute Li administration was able to overcome the reduction in response produced by DMI. It therefore appears unlikely that the potentiative interaction observed clinically has its basis in effects on the inositol phosphate second messenger system.

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