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BRIEF COMMUNICATION

Prevention of Fluphenazine-Induced Changes in Dopaminergic and Muscarinic Receptors by Lithium

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GIANUTSOS, G AND E. FRIEDMAN *Prevention of fluphenazine-induced changes in dopaminergic and muscarinic receptors by lithium* PHARMACOL BIOCHEM BEHAV 26(3) 635–637, 1987 —Co-administration of lithium carbonate to mice through their diet prevented the increase in dopamine receptor binding and the decrease in muscarinic receptor binding normally produced in striatum by chronic (12 week) administration of the neuroleptic, fluphenazine The lithium treatment failed to prevent the muscarinic down-regulation induced by the muscarinic agonist pilocarpine in the hippocampus These results (along with previous data) suggest that fluphenazine-induced striatal muscarinic receptor down-regulation and its reversal by lithium may be the consequence of changes in dopamine receptors in this brain region

Receptors Dopamine Neuroleptics Lithium Muscarinic

THE best recognized alteration accompanying the chronic administration of neuroleptic drugs is the development of supersensitivity of dopamine (DA) receptors (see [9] for review). More recently, changes in muscarinic cholinergic receptor binding have also been reported [4]. It has been suggested that these receptor changes may be responsible for the neurological disturbances accompanying chronic neuroleptic therapy, especially the development of tardive dyskinesia Consequently, drugs which may prevent or reverse these receptor changes have both pharmacologic and potential clinical relevance

The administration of lithium has been reported to prevent behavioral signs of DA supersensitivity (see [3] for review), although some studies have failed to provide corresponding biochemical support for this effect (i.e., prevention of the increase in binding sites for DA ligands). We chose to investigate the effects of lithium on the changes in DA and muscarinic receptors produced by long-term administration of fluphenazine in an attempt to further characterize the effects produced by lithium.

METHOD

Male mice (CD-1; Charles River Farms, Wilmington, MA) were housed 8 per cage. The mice received a standard ground mice diet (Purina) or ground chow containing 0.3 % (w/w) lithium carbonate. After 1 week on these diets, half the mice in each group received fluphenazine (0.005% w/v, [4]) dissolved in their drinking water, while the other half continued to receive unadulterated water. These treatments were carried out for an additional 12 weeks At the end of this period, the drug-containing bottles were replaced with plain water, treated mice did not differ significantly in weight from controls at the conclusion of the study. The mice were sacrificed 24 hours later; plasma lithium levels in the treated groups were in the normal therapeutic range (0 89±0.10 mEq/liter, N=16)

A separate group of mice were maintained on normal or lithium-containing chow and received pilocarpine (0.1% W/V) in their drinking water for 12 weeks. The mice also received methyl atropine (0 025%) to reduce peripheral muscarinic activity.

The binding of spiroperidol and QNB to striatal tissue was performed as previously described [4]. In brief, tissue was homogenized in 50 mM phosphate buffer (pH=7.4) and the pellet obtained after centrifugation at 37,000 × g for 10 min was resuspended and aliquots taken for the receptor binding assay. For ^{3}H-QNB, (New England Nuclear) various concentrations (for Scatchard analysis) or 0.5 nM was incubated for 1 hr at 25°C; non-specific binding was determined in the presence of 4 μM scopolamine. For DA binding, 1 nM ^{3}H-spiroperidol (New England Nuclear) was incubated in the

TABLE 1

EFFECT OF LITHIUM ON FLUPHENAZINE (FLU)-INDUCED CHANGES IN STRIATAL DOPAMINE AND MUSCARINIC BINDING*

Ligand	Total Binding (pmoles/mg protein, mean ± SE) Control	FLU	Lithium	Li + FLU
Spiroperidol	1 21 ± 0 08	1 54 ± 0 05†	1 14 ± 0 09	1 18 ± 0 05
QNB	2 59 ± 0 13	2 21 ± 0 09†	2 59 ± 0 19	2 61 ± 0 13

*Mice received lithium and fluphenazine for 13 and 12 weeks, respectively, as described in text Values are total binding in striata at 1 concentration of spiroperidol (1 nM) or QNB (0 5 nM) and are means obtained from 5 mice

†Denotes values significantly different from control ($p<0$ 05)

TABLE 2

EFFECT OF LITHIUM ON PILOCARPINE (PILO)-INDUCED CHANGES IN HIPPOCAMPAL MUSCARINIC BINDING*

Treatment	QNB Binding (Mean ± SE) Bmax	Kd
Control	1 85 ± 0 013	0 053 ± 0 004
Methyl Atropine (MA)	1 78 ± 0 10	0 056 ± 0 004
PILO + MA	1 34 ± 0 08†	0 073 ± 0 005‡
Li + PILO + MA	1 25 ± 0 08	0 076 ± 0 008

*Mice were treated with MA and/or PILO for 12 weeks and with lithium for one additional week preceding PILO treatment Binding parameters were determined in 5 mice per group and the means ± SEM are presented Bmax is expressed as pmoles/mg protein while Kd is nM

†‡Denote significant difference in comparison to both control and MA treatment (†=$p<0$ 01, ‡=$p<0$ 025)

presence of ketanserin to eliminate binding to serotonin receptors, non-specific binding was determined in the presence of 10 μM sulpiride The single concentrations of labelled ligands are near saturating conditions as previously determined. Student's t-test (two-tailed) was used for statistical analysis of the data

RESULTS

The effect of the long-term drug administration on striatal spiroperidol (DA ligand) and QNB (muscarinic ligand) binding is summarized in Table 1. Spiroperidol binding increased while QNB binding decreased after 12 weeks of fluphenazine treatment Lithium by itself had no effect on either binding site However, co-administration of lithium prevented both the spiroperidol up-regulation and the down-regulation of QNB binding normally induced by fluphenazine

The effect of long-term administration of pilocarpine on QNB binding in the hippocampus is summarized in Table 2 The pilocarpine treatment reduced the number of QNB binding sites in the hippocampus and significantly increased the Kd. Co-administration of lithium failed to prevent the effects of pilocarpine. Similar results were observed in the cortex (data not shown)

DISCUSSION

These results demonstrate that co-administration of lithium prevents the increase in DA receptor binding and the decrease in muscarinic cholinergic binding produced by long-term administration of fluphenazine. A number of other studies have shown that lithium prevents the behavioral supersensitivity to DA agonists resulting from chronic haloperidol [10,12] We also observed (data not shown) that the lithium treatment prevented the increase in apomorphine-induced cage climbing behavior and the tolerance to fluphenazine-induced catalepsy associated with chronic treatment (see also [2])

Most studies, however, have failed to provide a biochemical basis for the reduction in the behavioral supersensitivity While Pert and coworkers [10] reported that lithium prevented the haloperidol-induced increase in spiroperidol labelled binding sites, efforts to replicate these results have been, heretofore, unsuccessful (e g , [11,12])

The reasons for these disparate results are quite speculative, and include differences in species, dosing regimens and drugs In our study, mice were used, while all other published studies used rats Furthermore, our study continued for 84 days (since this is necessary to induce the muscarinic changes [4]), while other studies typically treated animals for 21 days. In addition, we produced changes with the phenothiazine, fluphenazine, while all of the previous research was performed with haloperidol Finally, it is interesting to note that both we and Pert and coworkers [10] used the carbonate form of lithium, while the studies which failed to produce biochemical changes used lithium chloride. Further research is needed to sort out the possible contribution of one or more of these variables

Of greater interest is the mechanism for the effect of lithium on both receptor types and its significance in understanding neuroleptic-induced neurological changes. We failed to observe any effect of lithium alone on either striatal spiroperidol binding or striatal or hippocampal QNB binding in agreement with previous reports [8,10], although some studies have reported an increase in striatal (but not hippocampal) QNB binding [6] or in whole forebrain QNB binding [5] in rats receiving lithium

Our previous study [4] suggests that the alteration in striatal QNB binding produced by fluphenazine is an indirect effect [4] mediated by disinhibition of the dopaminergic regulation of striatal cholinergic neurons. Lithium could, therefore, act directly on the muscarinic receptor to reduce its

plasticity or could act by preventing the dopaminergic receptor changes and thereby, indirectly, cancel out effects on the cholinergic neuron The results of Levy and coworkers [7], and those presented here (Table 2) would argue against the former mechanism since they suggest that lithium did not alter muscarinic receptor down-regulation in whole brain (induced by an inhibitor of cholinesterase), or in the hippocampus and cerebral cortex (induced by the directly acting cholinergic agonist pilocarpine). The possibility that lithium may exert effects on acetylcholine release [6] also remains to be explored

In summary, lithium prevented both the dopaminergic and cholinergic receptor changes induced by long-term fluphenazine administration in the striatum Although the exact mechanism responsible for this effect is not known, it is interesting that the muscarinic changes induced by fluphenazine were altered while the direct effects of pilocarpine on muscarinic receptors in non-dopaminergic areas were not. This suggests that certain receptor changes are sensitive to lithium while others are not (see also, [1]) and that these different sensitivities must be considered in assessing the results of behavioral studies.

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