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SYNERGISM OF THE TOXICITY OF PHYSOSTIGMINE AND NEOSTIGMINE BY LITHIUM OR BY A RESERPINE-LIKE AGENT (Ro4-1284)*

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SUMMARY

A single sublethal i.p. dose of lithium chloride (300 mg/kg or 7.1 meq/kg) followed 12 h later by an otherwise sublethal s.c. dose of physostigmine sulfate (1.0 mg/kg) resulted in 90% mortality among male rats following a pronounced cholinergic syndrome, including convulsions. This confirms a previous report of a lethal synergism of physostigmine after subacute dosing with lithium. Mortality could be completely prevented by 1.0 mg/kg of atropine sulfate given 30 min before physostigmine, but was incompletely, if at all, reduced by selective peripheral cholinergic blockers, methylatropine bromide (0.5, 1.5 mg/kg) or glycopyrrolate (1 mg/kg). This suggested a predominantly central site for the toxic interaction. However, a similar synergism of lethality caused by neostigmine methylsulfate (0.3 mg/kg, s.c.) after treatment with lithium, which could be eliminated by methylatropine or glycopyrrolate, indicates that lithium may also produce lethal synergism of a cholinesterase (ChE) inhibitor that does not act centrally. Ro4-1284, an agent that has reserpine-like actions, was tested in combination with physostigmine or neostigmine; it showed synergism of toxicity nearly the same as in the case of lithium plus the cholinergic agents. These findings support the hypothesis that lithium causes the toxic synergism via a reduction of adrenergic activity, leading to an imbalance between adrenergic and cholinergic influences and a consequent failure to tolerate the effects of the ChE inhibitors. A potential hazard for the clinical use of physostigmine and neostigmine, concurrently with lithium or reserpine-like agents, it suggested.

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INTRODUCTION

Numerous reports of the successful use of physostigmine to antagonize delerium, somnolence or coma resulting from accidental intoxicating doses of anticholinergics [1], tricyclic antidepressants [1,2], antiparkinson drugs [3], neuroleptic phenothiazines [4] and benzodiazepines [5] have been recorded. These have in turn encouraged the use of physostigmine to reverse similar adverse, unwanted effects of anticholinergics and anesthetics in the post-surgical period [6—9] as well as excessive sedative or other effects of therapeutically administered benzodiazepines [10—11] and neuroleptics [12].

In view of the evidently increasing acceptance of physostigmine for such non-emergency uses in anesthesiology, a recognition of conditions that might increase the hazard of using physostigmine is important. Samples et al. [13] recently described in brief one such condition, the lethal synergism of an acute dose of physostigmine by the action of prior subacute dosing with lithium. We report here confirmation and extension of the former study to provide further insight into the nature and mechanism of this interaction.

METHODS AND MATERIALS

Adult male rats (400—500 g) of the Sprague—Dawley strain (Harlan Laboratories, Cumberland, Indiana) were utilized. Rather than the 4.5-day dosing schedule of an earlier report [13], we tested whether the synergism would also occur when physostigmine sulfate (1.0 mg/kg) was given 12 h after a single dose of lithium chloride (300 mg/kg). The dose of lithium chosen was expected to be safe as Samples et al. [13] gave 100 mg/kg twice daily for 4.5 days, and Shimomura et al. [14] gave 250 mg/kg daily for 4 days. The lithium dose was given at 9 : 00—9 : 30 p.m., i.e., during the dark phase (2000—0800 h) of the rats' daily light cycle. Lithium treatment was given intraperitoneally (i.p.), while physostigmine was always given subcutaneously (s.c.). Volumes of injection were 1.0 ml/kg. Symptomatic observations of the rats were frequent during the first 4 h after physostigmine; a mortality count was obtained at 2, 6, 12, 24 and 48 h after physostigmine. Statistical analysis of mortality data for variation between experimental groups was by means of the Fisher Exact Probability Test or Chi-square Test [15].

Effects on toxicity of the combination of lithium and physostigmine were determined for atropine sulfate (1.0 mg/kg) and methylatropine bromide (0.5 mg/kg), both injected s.c. In a further study, s.c. doses of methylatropine at 1.5 mg/kg and glycopyrrolate at 1.0 mg/kg were also tested. In each case the anticholinergic preceded the cholinergic agent by 30 min. Male Long-Evans rats were also treated with lithium and physostigmine as above to test for synergism in another strain.

The interaction of lithium with neostigmine methylsulfate (0.1 and 0.3 mg/kg, s.c.) was tested in a parallel fashion to physostigmine. Again,

methylatropine and glycopyrrolate were tested as in the physostigmine studies for an ability to reduce mortality.

A short-acting reserpine-like agent [16], Ro4-1284 (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxyl-1,2,3,4,6,7-hexahydro-11*bH*-benzo[*a*]quinolizine), was administered in a s.c. dose of 10 mg/kg at 30 min before physostigmine or neostigmine and 15 min before atropine or methylatropine. Control groups received equivalent volumes of saline at corresponding times in place of 1 or both of the other 2 drug classes in the various combinations.

The sources of drugs used were: lithium chloride, J.T. Baker Chem. Co.; physostigmine sulfate, Sigma Chem. Co; neostigmine methylsulfate and Ro4-1284, Hoffmann-LaRoche; atropine sulfate, Merck; methylatropine bromide, Mann Research Laboratories; glycopyrrolate, A.H. Robins.

RESULTS

No deaths were seen following the presently-used doses of lithium or physostigmine alone (Table I); however, near-total mortality resulted from physostigmine in lithium-pretreated rats (Table IC, F). Whereas this dose of physostigmine alone produced only minimal external signs, slight salivation and increased defecation, there was a pronounced cholinergic symptomatology after lithium plus physostigmine, including strong salivation, tremors, and moderate to strong clonic convulsions. Severe convulsiform leaping also

TABLE I

MORTALITY OF MALE SPRAGUE—DAWLEY RATS AFTER LITHIUM CHLORIDE (i.p.) AND PHYSOSTIGMINE SULFATE (s.c.)

Treatments and dosages (mg/kg)[a]	No. dead/No. treated	Percent mortality
A. Lithium Cl (300) + saline	0/10	0
B. Saline + physostigmine (1.0)	0/10	0
C. Lithium Cl (300) + physostigmine (1.0)	19/21	90[b]
D. Lithium Cl (300) + atropine (1.0) + physostigmine (1.0)	0/11	0
E. Lithium Cl (300) + methylatropine (0.5) + physostigmine (1.0)	6/11	55[c]
F. Lithium Cl (300) + methylatropine (1.5) + physostigmine (1.0)	8/10	80[d]
G. Lithium Cl (300) + glycopyrrolate (1.0) + physostigmine (1.0)	8/10	80[d]

[a]Lithium treatment always preceded the physostigmine by 12 h; atropine, methylatropine or glycopyrrolate preceded physostigmine by 30 min.

[b]Significantly elevated ($P < 0.005$) from groups A, B or D.

[c]Significantly elevated ($P = 0.01$) from groups A, B or D and significantly ($P < 0.01$) lower than group C.

[d]Significantly elevated ($P < 0.01$) from groups A, B or D, but N.S. compared to group C.

occurred in repeated episodes lasting up to 4 h. The earliest deaths were at about 90 min; 5 out of 10 deaths had occurred by 12 h, 3 by 24 h, and the remaining 2 deaths between 24 and 48 h.

The incidence of deaths when either 0.5 or 1.5 mg/kg of methylatropine, was given 30 min before physostigmine was not significantly less than with only the lithium pretreatment. The same outcome also obtained for another peripherally-acting anticholinergic, glycopyrrolate (Table I). Convulsions still occurred, but they appeared to be less pronounced. In contrast, both mortality and convulsions produced by the lithium-physostigmine combination were completely abolished by atropine. Mortality after combined sublethal doses of lithium and physostigmine in Long-Evans rats was identical (91%) to that for the Sprague—Dawley rats, so the interaction is not peculiar to a single strain.

No evidence for synergism was noted when a sublethal dose (0.1 mg/kg) of neostigmine methylsulfate was given 12 h after lithium chloride (Table II). However, when the test was repeated with 0.3 mg/kg of neostigmine, which alone produced only 10% mortality, there were significant increases after lithium to a 60% or 75% mortality (Table IID, G). Whereas atropine completely prevented deaths from lithium and neostigmine, there was still 1 death in each case from the combination when both methylatropine and glycopyrrolate were tested in place of atropine (Table II). Neostigmine toxicity developed more rapidly and convulsions were more continuous and severe than with physostigmine, but they were of shorter duration as all deaths occurred within the first 30 min. Chromodacryorrhea was more severe after neostigmine than with physostigmine.

TABLE II

MORTALITY OF MALE SPRAGUE—DAWLEY RATS AFTER TREATMENT WITH LITHIUM CHLORIDE (i.p.) AND NEOSTIGMINE METHYLSULFATE (s.c.)

Treatments and dosages (mg/kg)[a]	No. dead/No. treated	Percent mortality
A. Saline + neostigmine (0.1)	0/10	0
B. Lithium Cl (300) + neostigmine (0.1)	0/10	0
C. Saline + neostigmine (0.3)	1/10	10
D. Lithium Cl (300) + neostigmine (0.3)	6/10	60[b]
E. Lithium Cl (300) + atropine (1.0) + neostigmine (0.3)	0/10	0
F. Lithium Cl (300) + methylatropine (0.5) + neostigmine (0.3)	1/10	10
G. Lithium Cl (300) + saline + neostigmine (0.3)	8/12	75
H. Lithium Cl (300) + glycopyrrolate (1.0) + neostigmine (0.3)	1/11	9[c]

[a]Lithium treatment always preceded neostigmine by 12 h; atropine, methylatropine or glycopyrrolate preceded neostigmine by 30 min.
[b]Significantly elevated from groups C and F ($P < 0.05$) and E ($P < 0.01$).
[c]Significantly lower than groups G ($P < 0.01$).

TABLE III

MORTALITY OF MALE SPRAGUE—DAWLEY RATS AFTER Ro4-1284 (s.c.) AND PHYSOSTIGMINE (s.c.) OR NEOSTIGMINE METHYLSULFATE (s.c.)

Treatments and dosages (mg/kg)[a]	No. dead/No. treated	Percent mortality
A. Ro4-1284 (10) + saline	0/10	0
B. Ro4-1284 (10) + physostigmine (1.0)	8/10	80[b]
C. Ro4-1284 (10) + atropine (1.0) + physostigmine (1.0)	0/10	0
D. Ro4-1284 (10) + methylatropine (0.5) + physostigmine (1.0)	2/10	20[c]
E. Ro4-1284 (10) + neostigmine (0.3)	7/10	70[d]
F. Ro4-1284 (10) + atropine + neostigmine (0.3)	0/10	0
G. Ro4-1284 (10) + methylatropine (0.5) + neostigmine (0.3)	0/10	0

[a]Ro4-1284 treatment always preceded physostigmine or neostigmine by 30 min; atropine or methylatropine was given at 15 min after Ro4-1284.
[b]For B vs. A or C, $P < 0.001$.
[c]For D vs. B, $P = 0.025$.
[d]For E vs. A, F or G, $P = 0.005$.

The combination of Ro4-1284 with physostigmine or neostigmine produced nearly the identical degree of mortality as for the former combinations including lithium instead (Table III). Moreover, the effects of adding atropine or methyltropine to test the 3-way interaction also gave very similar results. Namely, atropine completely blocked all mortality from either cholinesterase inhibitor, as methylatropine also did with neostigmine; however, methylatropine provided a large but not total antagonism of mortality after physostigmine plus Ro4-1284. In preliminary observations, it was noted that the lethal synergism of Ro4-1284 with neostigmine and physostigmine was considerably less if the interval between the drugs was lengthened from 30 min to 120 min. At the latter interval, mortality was 30% in each case rather than 70% and 80%. Therefore, only the shorter interval was used to give optimal results for the present purposes.

DISCUSSION

These results provide confirmation for an earlier report [13] of a lethal interaction between lithium and physostigmine in rats. The synergism was even more strongly evident after our single-dose lithium treatment than was the 80% mortality found by Samples et al. [13] after 9 doses of lithium over 4.5 days. When their largest daily dosage (200 mg/kg divided between 2 equal doses at a 12-h interval) was given but once before physostigmine, the resulting mortality was about 40%. Thus, a toxic synergism with physostigmine occurs in rats after either acute or subacute dosing with lithium.

While the earlier study [13] showed that the enhanced effect of physostigmine could be reversed by an antimuscarinic agent, scopolamine HBr, it did not distinguish between a peripheral or a central site of action. As we found that atropine was quite effective in abolishing mortality although methylatropine was ineffective, it could be inferred that the toxic action occurs predominantly via actions at the level of the central nervous system. This conclusion at first appeared to be further supported by a failure to detect synergism between lithium and a sublethal 0.1 mg/kg dose of neostigmine, a cholinesterase inhibitor which has little or no CNS effect [17]. However, when a larger dose (0.3 mg/kg) was tested, a synergism with prior lithium treatment occurred and could be prevented by means of anticholinergics that act peripherally but not centrally. Thus the toxic interaction may not be attributed entirely to a central mechanism.

In view of the results discussed above, we favored the interpretation given for their results by Samples et al. [13], that the magnification of physostigmine toxicity after lithium occurs because of "a shift in adrenergic-cholinergic balance to cholinergic predominance". This could occur because the adrenergic component is made deficient through the action of lithium either to inhibit catecholamine synthesis or otherwise reduce its functional availability [18—20]. Consequently, the lithium-treated organism may become less able to compensate for the enhancement of the cholinergic component produced by a cholinesterase inhibitor such as physostigmine. To test this interpretation, further experiments were performed using Ro4-1284 to reduce CNS adrenergic functions via an action like that of reserpine [16]. The results of these were so similar to those with lithium as to provide support for the above-stated interpretation. Furthermore, the results with Ro4-1284 and the cholinesterase inhibitors were in accord with previous data for mice showing a lethal interaction of reserpine with physostigmine or neostigmine [21]. The finding that the diverse actions of lithium and Ro4-1284 (each reducing central adrenergic activity by a different mechanism) apparently are correlated with a lethal synergism of the 2 cholinesterase inhibitors supports such a common effect as the mechanism for the synergism. These results also point to the same potential for hazardous interaction between reserpine (or like drugs) and cholinesterase inhibitors, as was suggested previously [21].

While it is not possible to equate this single-dose treatment with lithium to a human therapeutic regimen, it should be noted that the dosage employed (300 mg/kg or 7.1 meq/kg) caused no overt toxicity. No neurological abnormalities, and only mild lethargic symptoms, could be seen in our rats, as was also true in others' observations for a higher dosage (10 meq/kg) for 3 daily doses [22].

The importance of a medication history for patients to whom physostigmine or neostigmine is to be administered is emphasized by these experimental findings. This may not be available in the case of emergency applications of physostigmine; however, it should be when these agents are used in anesthesiology. It is of significance that 2 recent reviews [23,24] did not

include lithium and cholinesterase inhibitors as potential hazards for drug interactions, although each reported a significant prolongation by lithium of the neuromuscular blocking action of succinylcholine, decamethonium and pancuronium in human patients. With awareness of possible hazards of such combinations, serious clinical problems may be avoided.

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