Haloperidol attenuation of morphine abstinence: synergistic effect of acute lithium administration

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Acute lithium (Li) carbonate administration has been reported to potentiate the suppressive effect of subthreshold doses of haloperidol on food-reinforced operant behaviour in rats (Ahlenius & Engel, 1974). Since a behaviourally-inactive dose of α-methyltyrosine (α-MT), a tyrosine hydroxylase inhibitor, also potentiates the suppressive effect of haloperidol (Ahlenius & Engel, 1971) as well as similar effects of very low doses of other neuroleptics (Ahlenius & Engel, 1973; Engel, 1975), one suggestion made by these authors for this potentiation is that Li may inhibit a compensatory feedback mechanism that is responsible for increased catecholamine synthesis during receptor blockade by neuroleptics (Carlsson, Persson & others, 1972; Carlsson, Roos & others, 1973). α-MT also potentiates neuroleptic suppression of tail-pinch-induced eating (Antelman, Szechtman & others, 1976).

We assessed the effect of acute Li and haloperidol administration by use of a narcotic abstinence model, since neuroleptics (Puri & Lal, 1973; Lal & Numan, 1976), as well as catecholamine depletion (Schwartz & Eidelberg, 1970; Glick, Zimmerberg & Charap, 1973; Herz, Blasig & Papeschi 1974), can suppress or decrease morphine abstinence signs. We report that acute Li had no effect itself, but significantly increased the abstinence-attenuating effect of haloperiodol. However, since α-MT did not exert similar synergistic action with haloperidol at α-MT doses effective with neuroleptics in suppressing operant behaviour (Ahlenius & Engel, 1971, 1973), it is unlikely that the effect of Li in the present report can be attributed specifically to an α-MT-like action on tyrosine hydroxylase.

Male, Sprague-Dawley, Charles River CD rats, 160–210 g, were implanted with a 75 mg morphine pellet (Gibson & Tingstad, 1970) under ether anaesthesia. At 72 h, abstinence was precipitated by a 4 mg kg^{-1} (i.p.) injection of naloxone HCl. Scoring of abstinence was based on a system modified from that of Wei (1973), such that each animal's abstinence score was the total number of signs (maximum of nine) exhibited during the 15 min observation period (Hine, Friedman & others, 1975), which included wet shakes, escapes, faecal boluses, diarrhoea, vocalization, abnormal posture, ear blanching, ptosis, teeth chattering and mouth movements.

Three experiments were performed. Expt. 1 attempted to determine effects on morphine abstinence of acute doses of Li, haloperidol, and their combination that have produced suppression of operant behaviour (Ahlenius & Engel, 1974). Animals received either 100 mg kg^{-1} Li_2CO_3 (adjusted to pH 7) or saline 4 h before naloxone (this Li dose resulted in plasma concentrations of 0·82 ± 0·06 mequiv $litre^{-1}$ in a separate group of 6 rats as determined by atomic absorption spectrophotometry). Subgroups of these animals received 0·04 mg kg^{-1} haloperidol or saline 15 min before naloxone. Data from 7–8 animals per subgroup can be summarized in the following manner. Alone, Li or haloperidol had no effect on abstinence scores. There were a large number of spontaneous and post-naloxone escapes in the Li group, possibly reflecting a hyperexcitable state. The combination of Li and this dose of haloperidol resulted in a significantly reduced abstinence score. However, three additional replications of this effect were attempted without success. That is, when data from a saline control group were compared with data from three other groups receiving Li and haloperidol as described, abstinence scores were not significantly modified, although a statistical reduction of faecal boluses occurred in one group.

Usually, doses of haloperidol slightly more than ten times greater than that used above are necessary to significantly modify abstinence scores as defined under the conditions of our experiments (Hine & others, 1975), and similar doses are within the range of those where decreases in morphine withdrawal and the antagonism of morphine self-administration are dose-related (Glick & Cox, 1975; Lal & Numan, 1976). Interactions between haloperidol, in doses of 0·5–1·0 mg kg^{-1}, and Li_2CO_3 were further assessed in Expt. 2, and these data are shown in Table 1. Here, a Li_2CO_3 dose of 200 mg kg^{-1} (resulting in plasma concentrations of 2·23 ± 0·07 m equiv $litre^{-1}$) was used. At the time of testing, these lithium values produced less hyperexcitability than the previous dose, produced no sedation or other obvious signs of toxicity (possibly due to neutralization of the carbonate to near-physiological pH before injection: Baldessarini & Yorke, 1970), have been reported to produce Li brain concentrations of 0·6–0·75 m equiv $litre^{-1}$ in rats (Frazer, Mendels & others, 1973), do not modify brain dopamine synthesis in the strain and weight of rats used here when given acutely (Friedman & Gershon, 1973), and, as shown in Table 1 did not modify abstinence scored in the Li + Sal group.

Table 1 also indicates that although abstinence scores were not affected by Li, faecal boluses were decreased, and there was an increased tendency toward post-naloxone escapes. The synergistic effect of Li on an otherwise sub-threshold abstinence-modifying dose of haloperidol (0·5 mg kg^{-1}) in decreasing abstinence was

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Table 1. *Mean (±s.e.m.) values for abstinence signs in morphine-dependent rats pretreated acutely with Li_2CO_3 and various doses of haloperidol.*

Group	n	Wet shakes	Escapes	Faecal boluses	Abstinence score
Sal + Sal	9	4·6 ± 0·8	3·4 ± 0·7	10·8 ± 1·4	7·2 ± 0·3
Li + Sal	9	3·2 ± 0·7	6·8 ± 1·7	7·9 ± 0·9*	6·8 ± 0·4
Sal + Hal (0·5)	8	4·4 ± 1·2	2·6 ± 0·9	9·4 ± 0·9	6·7 ± 0·4
Li + Hal (0·5)	7	1·8 ± 0·8*‡	4·6 ± 1·6	5·1 ± 1·0*‡	5·4 ± 0·8*
Sal + Hal (0·75)	9	2·6 ± 0·4*	0·8 ± 0·3*	11·2 ± 0·5	7·0 ± 0·4
Li + Hal (0·75)	9	0·2 ± 0·1*‡	0·3 ± 0·2*	5·0 ± 0·2*‡	5·1 ± 0·5*‡
Sal + Hal (1·0)	9	2·4 ± 0·6*	2·6 ± 0·9	6·9 ± 1·0*	5·5 ± 0·6*
Li + Hal (1·0)	8	1·0 ± 0·4*	1·2 ± 0·4*	4·8 ± 0·4*	4·8 ± 0·6*

† Haloperidol (Hal) mg kg^{-1} doses in parentheses. All rats implanted with a morphine pellet and abstinence precipitated 72 h later with 4 mg kg^{-1} naxolone HCl. Li_2CO_3 (Li) at 200 mg kg^{-1} or saline (Sal) were given i.p. 4 h before naloxone; Hal or Sal were injected 15 min before naloxone.
* Significant ($P < 0.05$) Mann-Whitney comparisons with respective Sal + Sal control group, or ‡ with immediately preceding Sal + Hal group.

clearly demonstrated for wet shakes, faecal boluses, and total abstinence score. Moreover, acute Li significantly increased the suppressive effect of 0·75 mg kg^{-1} haloperidol on wet shakes, faecal boluses, and abstinence scores. This effect of Li was still observable, even with the marked suppression of abstinence produced by haloperidol alone at a dose of 1·0 mg kg^{-1}.

To determine whether the synergistic effect of acute Li could be mimicked by use of α-MT, Expt. 3 was performed. Using the same design as in Expt. 2, Li_2CO_3 was replaced with α-MT methyl ester in acute doses of 50 or 100 mg kg^{-1}. This procedure, although resulting in lower cumulative α-MT doses than those previously reported effective in attenuating morphine abstinence signs in rats (e.g. Herz & others, 1974), depletes brain catecholamines sufficiently at 4 h to produce significant impairment in shock-avoidance behaviour (Rech, Borys & Moore, 1966), and has completely suppressed operant behaviour, in combination with very low doses of haloperidol (Ahlenius & Engel, 1971, 1973). The data of Table 2 indicate that the combination of α-MT + haloperidol was no more effective than haloperidol alone in attenuating abstinence. No effect of α-MT was observed on escapes (not shown), but there was a trend toward an elevation of wet shakes by α-MT alone, an effect which has been observed by Herz & others (1974) only in highly morphine dependent rats.

Table 2. *Mean (±s.e.m.) values for abstinence signs in morphine-dependent rats pretreated acutely with α-methyl-*p*-tyrosine (α-MT) and haloperidol.*†

Group	n	Wet shakes	Faecal boluses	Abstinence score
Sal + Sal	7	4·7 ± 0·8	9·6 ± 0·7	7·4 ± 0·4
Sal + Hal (0·50)	8	2·8 ± 0·6*	8·8 ± 0·8	6·5 ± 0·8
α-MT (50) + Sal	7	6·7 ± 1·4	9·1 ± 0·9	6·9 ± 0·4
α-MT (100) + Sal	7	6·0 ± 0·6	8·9 ± 0·9	7·7 ± 0·4
α-MT (100) + Hal (0·50)	7	2·1 ± 0·7*	6·8 ± 0·9*	6·2 ± 0·9
α-MT (100) + Hal (0·75)	8	1·8 ± 0·5*	6·1 ± 1·2*	6·1 ± 0·3*

† Haloperidol (Hal) and α-MT doses in parentheses. α-MT methyl ester or saline (Sal) injected i.p. 4 h before naloxone HCl (4 mg kg^{-1}). Hal or Sal injected i.p. 15 min before naloxone.
* Significant ($P < 0.05$) Mann-Whitney comparisons with Sal + Sal group.

These data, then, demonstrate a synergistic effect of acute Li on attenuation of morphine abstinence by haloperidol that is probably not due to an α-MT-like effect of Li on catecholamine synthesis under conditions of present experiments. Indeed, Segal, Callaghan & Mandell (1975) have reported an *increase* in caudate and striatal tyrosine hydroxylase activity after chronic Li in male rats, possibly reflecting a compensatory response to Li-induced modification of catecholamine function (c.f. Schildkraut, 1974). While such speculation could be relevant to possible mechanisms underlying a reported reduction in morphine self-administration during chronic Li treatment (Tomkiewicz & Steinberg, 1974) an adequate explanation for the acute synergistic effect of Li with haloperidol reported here requires further research.

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Piperazic acid and related compounds as inhibitors of GABA uptake in rat brain slices

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Cellular uptake of GABA by specific transport processes may have an important physiological role at synapses which use GABA as a neurotransmitter. There is, therefore, a continuing interest in inhibitors of GABA uptake which may be useful pharmacological agents. Two relatively selective inhibitors that have been studied extensively are 2,4-diamino-butyric acid and nipecotic acid (Iversen & Kelly, 1975; Krogsgaard-Larsen & Johnston, 1975). As illustrated in Fig. 1, these inhibitors are structurally related to piperazic acid (hexahydropyridazine-3-carboxylic acid), derivatives of which occur in the monamycins, a family of cyclodepsipeptide antibiotics obtained from cultures of *Streptomyces jamaicensis* (Hassall, Morton & others, 1969; 1971). The optical isomers of piperazic acid, and some related compounds, have been examined as inhibitors of GABA uptake in rat brain slices and *S*(−)-piperazic acid found to be a potent inhibitor.

The sodium-dependent, "high-affinity" uptake of GABA (10 nM exogenous concentration) in "minislices" of rat cerebral cortex (0·1 × 0·1 × 2·0 mm) at 25° and the transamination of GABA catalysed by extracts of rat cerebral cortex were studied as described in detail elsewhere (Beart, Johnston & Uhr, 1972a; Beart, Uhr & Johnston, 1972b). The uptakes of L-glutamate (10 nM), β-alanine (0·3 nM) and L-proline (6 nM) were studied by similar procedures (Balcar & Johnston, 1972; Balcar, Johnston & Stephanson, 1976; Johnston & Stephanson, 1976). Unless stated otherwise, potential inhibitors were preincubated with the brain slices for 15 min.

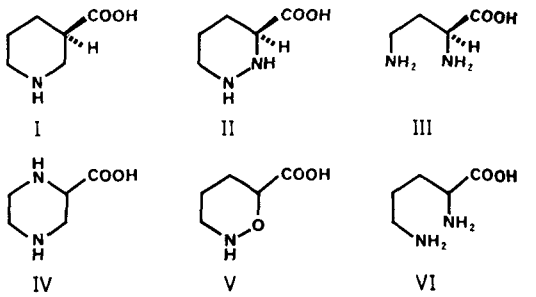

FIG. 1. Some structurally-related inhibitors of GABA uptake. I—*R*(−)-Nipecotic acid, II—*S*(−)-piperazic acid, III—*S*(+)-2,4-diamino-butyric acid, IV—(±)-piperidazine-2-carboxylic acid, V—perhydro-1,2-oxazine-6-carboxylic acid, VI—(±)-ornithine.

S(−)- and *R*(+)-Piperazic acid, and derivatives thereof, were gifts from Dr C. H. Hassall, Roche Products Ltd., Welwyn Garden City. (±)-Piperazine-2-carboxylic acid was prepared by catalytic hydrogenation of pyrazine-2-carboxylic acid (Felder, Maffei & others, 1960); it had a m.p. of 285° and analysed correctly for C, H, N.

The effects of *S*(−)- and *R*(+)-piperazic acid and of (±)-piperazine-2-carboxylic acid on GABA uptake are summarized in Table 1 and compared with the effects of some structurally related substances. *S*(−)-Piperazic acid was approximately 25 times as potent as the *R*(+)-stereoisomer, and was intermediate in potency between *R*(−)-nipecotic acid and *S*(+)-2,4-diamino-butyric acid. The 1-benzyloxycarbonyl derivative of *S*(−)-piperazic acid did not significantly inhibit GABA

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