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Actions of chlorpromazine, haloperidol and pimozide on lipid metabolism in guinea pig brain slices*

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Chlorpromazine causes an increase in the metabolism of phosphatidic acid and phosphatidylinositol in guinea pig and rat brain slices, as measured by the incorporation of various radioactive precursors [1, 2]. The metabolism of phosphatidylcholine and phosphatidylethanolamine is not increased. Several other cationic amphophilic drugs have similar effects in various tissues [3]. The effect appears to involve increased synthesis *de novo* of phosphatidic acid and of phospholipids such as phosphatidylinositol that are synthesized via the CDP-diacylglycerol pathway. In this respect it differs from the receptor-mediated effect of some neurotransmitters on the metabolism of phosphatidylinositol, in which there is an increased turnover of phosphate and inositol in the lipid, without a concomitant synthesis of the diacylglycerol moiety [4]. It has been suggested that the effects of chlorpromazine and other drugs on phospholipid metabolism may be related to the clinical mechanisms of action of the drugs [2, 3]. In the present work, the actions of chlorpromazine, haloperidol and pimozide were compared to determine whether effects of these drugs on phospholipid metabolism might be correlated with their neuroleptic activity. These three compounds are representative of the phenothiazine, butyrophenone and diphenylbutylpiperidine classes of drugs that are used clinically as neuroleptic agents. Their interactions with effects of norepinephrine and dopamine on phospholipid metabolism were also examined.

[2-^{3}H]Glycerol, [2-^{3}H]*myo*-inositol and [5-^{3}H]cytidine were from New England Nuclear (Boston, MA). [^{32}P]Orthophosphate was from Schwarz/Mann (Orangeburg, NY). Sources of drugs were: chlorpromazine, Smith, Kline & French Laboratories (Philadelphia, PA); haloperidol, McNeil Laboratories, Inc. (Fort Washington, PA); pimozide, Janssen Pharmaceutica (Beerse, Belgium); and L-norepinephrine and dopamine, Sigma Chemical Co. (St. Louis, MO).

Guinea pig cerebral cortex slices and corpus striatum slices were prepared and incubated in Krebs–Henseleit bicarbonate medium, pH 7.4, with 5 mM glucose and radioactive precursors and drugs for 1 hr, as described elsewhere [5]. Lipids were extracted either as described [5], or by the method of Bligh and Dyer [6]. Individual lipids were separated by chromatography on either silicic acid-impregnated paper [5], or ITLC-SA glass fiber sheets [7].

In cerebral cortex slices, during an incubation period of 1 hr with chlorpromazine or haloperidol in a 0.1 mM concentration, there was an increased incorporation of [2-^{3}H]glycerol into phosphatidylinositol, without any significant effect on the incorporation of this precursor into phosphatidylcholine or triacylglycerol (Table 1); these results suggest that the drugs promote an increased synthesis *de novo* of phosphatidylinositol. With chlorpromazine there was also an increased incorporation of [2-^{3}H]glycerol into phosphatidic acid and diacylglycerol; pimozide (0.1 mM) did not significantly affect the incorporation of [2-^{3}H]glycerol into any of these lipids (Table 1).

The mechanism by which chlorpromazine and other cationic drugs affect lipid metabolism is not clear. Brindley *et al.* [3] suggested that the effect may be produced through an inhibition of phosphatidate phosphohydrolase, leading to decreased formation of diacylglycerol from phosphatidic acid, and resulting in a redirection of lipid pathways to give increased synthesis of phosphatidylinositol and phosphatidylglycerol, which are formed from phosphatidic acid via the CDP-diacylglycerol pathway, and decreased synthesis of phosphatidylcholine, phosphatidylethanolamine and triacylglycerol, which are formed via the diacylglycerol pathway. The observation by Brindley and Bowley [8] that chlorpromazine inhibits phosphatidate phosphohydrolase in an enzyme preparation from rat liver provided some support for this possible mechanism. The effects of propranolol on phospholipid biosynthesis in the rat pineal

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Table 1. Incorporation of [2-^{3}H]glycerol into lipids in guinea pig cerebral cortex slices in the presence of 0.1 mM chlorpromazine, haloperidol or pimozide

	[2-^{3}H]glycerol radioactivity* (cpm/mg tissue)			
Lipid	Control	Chlorpromazine	Haloperidol	Pimozide
Phosphatidic acid	97 ± 13	142 ± 4†	117 ± 6	92 ± 2
Phosphatidylinositol	157 ± 22	341 ± 50†	237 ± 26†	168 ± 24
Diacylglycerol	71 ± 8	101 ± 11†	93 ± 13	80 ± 14
Phosphatidylcholine	93 ± 13	98 ± 14	110 ± 13	105 ± 20
Triacylglycerol	131 ± 10	104 ± 4	146 ± 8	133 ± 9

* Each value is the mean ± S.E. of six observations for controls and four observations with drug added.

† Values with drug, expressed as per cent of control value in the same experiment, show significant difference from 100 per cent ($P < 0.05$, *t*-test).

Table 2. Incorporation of [^{32}P]phosphate into phospholipids in cerebral cortex and corpus striatum slices in the presence of 0.1 mM chlorpromazine, haloperidol and pimozide

Phospholipid	[^{32}P]Phosphate radioactivity* (cpm/μg total lipid-P)			
	Control	Chlorpromazine	Haloperidol	Pimozide
Cerebral cortex				
Phosphatidic acid	57 ± 8	124 ± 31†	114 ± 21†	61 ± 13
Phosphatidylinositol	118 ± 5	222 ± 38†	308 ± 38†	106 ± 12
Phosphatidylinositol phosphate	106 ± 17	224 ± 43	303 ± 76†	124 ± 27
Phosphatidylinositol diphosphate	280 ± 61	387 ± 39	418 ± 77†	295 ± 50
Phosphatidylethanolamine	63 ± 14	84 ± 17	86 ± 17	60 ± 9
Phosphatidylcholine	128 ± 12	104 ± 15	132 ± 18	114 ± 16
Corpus striatum				
Phosphatidic acid	39 ± 3	86 ± 16†	92 ± 15†	55 ± 6
Phosphatidylinositol	53 ± 7	100 ± 18†	97 ± 17†	58 ± 7
Phosphatidylinositol phosphate	87 ± 24	151 ± 49†	183 ± 90†	102 ± 19
Phosphatidylinositol diphosphate	223 ± 34	208 ± 71	279 ± 88	227 ± 23
Phosphatidylethanolamine	61 ± 27	45 ± 14	54 ± 18	50 ± 10
Phosphatidylcholine	56 ± 7	59 ± 13	67 ± 12	74 ± 6

* Each value is the mean ± S.E. of four observations. The ^{32}P specific activity of the ATP of the tissue was not affected significantly by any of the three drugs.

† Values with the drug, expressed as per cent of the control in the same experiment, show significant difference from 100 per cent ($P < 0.05$, t-test).

gland have been studied in detail by Eichberg *et al.* [9], and have also provided some support. These authors concluded that an inhibition of phosphatidate phosphohydrolase, which would give an increase in available phosphatidic acid and a decrease in available diacylglycerol, could account, in part, for the changes in phospholipid biosynthesis that they observed. But, they pointed out, although this is a plausible explanation, it has not been proven that this is the principal mechanism by which these drugs alter phospholipid metabolism in the intact cell. In the results reported here, there is no evidence that the effects of chlorpromazine and haloperidol on phospholipid synthesis in brain slices can be accounted for by an inhibition of phosphatidate phosphohydrolase. Synthesis of phosphatidylcholine and triacylglycerol, as measured by [2-^{3}H]glycerol incorporation, was not inhibited, and there was a small but significant increase in the incorporation of [2-^{3}H]glycerol in the diacylglycerol pool in the presence of chlorpromazine (Table 1). It is, of course, possible that a decrease in labeling of diacylglycerol and of those lipids that are synthesized by the diacylglycerol pathway might occur at concentrations of chlorpromazine or haloperidol higher than those tested here.

Chlorpromazine or haloperidol at a 0.1 mM concentration led to an increased incorporation of [^{32}P]phosphate into phosphatidic acid and phosphatidylinositol in slices of either cerebral cortex or corpus striatum. In some cases, there was also a significantly increased incorporation of [^{32}P]phosphate into phosphatidylinositol phosphate and phosphatidylinositol diphosphate; incorporation of

Table 3. Effects of chlorpromazine, haloperidol and pimozide on [^{32}P]phosphate and [2-^{3}H]*myo*-inositol incorporation into phosphatidylinositol in the absence and presence of 1 mM *l*-norepinephrine (NE) in cerebral cortex slices

Drug added (0.1 mM)	Phosphatidylinositol radioactivity* (cpm/μg lipid-P)			
	[^{32}P]phosphate		[2-^{3}H]*myo*-Inositol	
	No NE	+ 1 mM NE	No NE	+ 1 mM NE
Control	118 ± 5	197 ± 26†	62 ± 3	86 ± 5†
Chlorpromazine	222 ± 38‡	246 ± 27	155 ± 24‡	132 ± 11
Haloperidol	308 ± 38‡	276 ± 23	169 ± 15‡	126 ± 15
Pimozide	108 ± 12	214 ± 35†	81 ± 8	91 ± 6

* Each value for [^{32}P]phosphate is the mean ± S.E. of four observations; each value for [2-^{3}H]*myo*-inositol is the mean ± S.E. of six observations.

† Values with *l*-norepinephrine, expressed as per cent of values without *l*-norepinephrine in the same experiment, show significant difference from 100 per cent ($P < 0.05$, t-test).

‡ Values with drug, expressed as per cent of control in the same experiment, show significant difference from 100 per cent ($P < 0.05$, t-test).

Table 4. Effects of chlorpromazine, haloperidol and pimozide on [2-^{3}H]*myo*-inositol incorporation into phosphatidylinositol in the absence and presence of 1 mM dopamine in corpus striatum slices

Drug added (0.1 mM)	Lipid [^{3}H]inositol* (cpm/μg total lipid-P)	
	No dopamine	+ 1 mM Dopamine
Control	55 ± 4	35 ± 4†
Chlorpromazine	110 ± 19‡	59 ± 5†
Haloperidol	124 ± 17‡	56 ± 7†
Pimozide	52 ± 5	32 ± 3†

* Each value is the mean ± S.E. of six observations.

† Values with dopamine, expressed as per cent of values without dopamine in the same experiment, show significant difference from 100 per cent ($P < 0.05$, *t*-test).

‡ Values with drug, expressed as per cent of control in the same experiment, show significant difference from 100 per cent ($P < 0.05$, *t*-test).

[^{32}P]phosphate into phosphatidylcholine and phosphatidylethanolamine was not affected significantly by the drugs (Table 2). Radioactivity in phosphatidylserine and sphingomyelin was comparatively low and was not estimated. Pimozide (0.1 mM) produced no significant changes in the incorporation of [^{32}P]phosphate into any of the phospholipids studied (Table 2).

The incorporation of [2-^{3}H]*myo*-inositol into phosphatidylinositol was increased in the presence of chlorpromazine or haloperidol. In cerebral cortex slices, incorporation of [2-^{3}H]*myo*-inositol was increased 42, 52 and 98 per cent, respectively, in the presence of 1 μM, 10 μM and 0.1 mM chlorpromazine (mean of two observations). With concentrations of haloperidol between 10 μM and 1 mM, the highest concentration tested, there was an average increase of 93 per cent in [2-^{3}H]*myo*-inositol incorporation. Within this concentration range, the degree of stimulation was not significantly different at different concentrations of haloperidol (based on the mean of three observations at each concentration); there was no significant difference in [2-^{3}H]*myo*-inositol incorporation with 3 μM haloperidol.

CDP-diacylglycerol is an intermediate in the pathway of phosphatidylinositol synthesis. An increase of 96 per cent (mean of two observations) was found in the incorporation of [5-^{3}H]cytidine into CDP-diacylglycerol in cerebral cortex slices in the presence of 0.1 mM chlorpromazine; no significant increase was observed with 1 μM or 10 μM chlorpromazine.

The effects of pimozide at concentrations between 0.1 μM and 0.1 mM on [2-^{3}H]glycerol and on [^{32}P]phosphate incorporation into lipids was tested and no significant effects were found. The lack of effect of pimozide on lipid metabolism argues against a major role for such an effect in the overall neuroleptic actions that are common to the three drugs tested here.

Chlorpromazine and haloperidol, but not pimozide, can block norepinephrine receptors [10, 11]. It was of interest, therefore, to see whether these agents would interact with the effects of norepinephrine. Phosphatidylinositol metabolism is stimulated by norepinephrine in guinea pig cerebral cortex slices [5], and the effect is the same as that of other receptor-mediated phospholipid effects in that the turnover of [^{32}P]phosphate and [2-^{3}H]*myo*-inositol is increased, without any change in the incorporation of [2-^{3}H]glycerol (M. Hokin-Neaverson, unpublished results). Cerebral cortex slices treated with 1 mM norepinephrine incorporated approximately 50 per cent more [^{32}P]phosphate and [2-^{3}H]*myo*-inositol into phosphatidylinositol than did controls. When 1 mM norepinephrine was added in the presence of either 0.1 mM chlorpromazine or 0.1 mM haloperidol, the increase in incorporation of these radioactive precursors was not significantly different from that observed with chlorpromazine or haloperidol alone; pimozide had no significant effects (Table 3). These results suggest that chlorpromazine and haloperidol blocked the receptor-mediated effects of norepinephrine on phosphatidylinositol metabolism, and that norepinephrine did not have any significant effect on the increased metabolism of phosphatidylinositol brought about by chlorpromazine and haloperidol. It is possible, however, that there may have been a partial inhibition of the effects of both norepinephrine and the other drugs by a non-receptor-mediated interaction.

Chlorpromazine, haloperidol and pimozide can all block dopamine receptors [11]. Their interactions with the effects of dopamine were studied in slices of guinea pig corpus striatum, because this brain area is rich in dopaminergic nerve endings. In corpus striatum slices, 1 mM dopamine leads to inhibition of the metabolism of all phospholipids [12]. This inhibition, measured by the incorporation of [2-^{3}H]*myo*-inositol into phosphatidylinositol, was not counteracted by 0.1 mM pimozide (Table 4). It is unlikely, therefore, that this inhibition by dopamine was receptor-mediated. The stimulation of [2-^{3}H]*myo*-inositol incorporation by 0.1 mM chlorpromazine or haloperidol was reduced significantly in the presence of 1 mM dopamine (Table 4). The inhibition by dopamine appears, therefore, to have occurred at a step in phospholipid metabolism which interferes with the increased metabolism of phosphatidylinositol evoked by chlorpromazine or haloperidol.

In summary, both chlorpromazine and haloperidol stimulated the synthesis of phosphatidylinositol, that was measured by the incorporation of [2-^{3}H]glycerol, [^{32}P]phosphate and [2-^{3}H]*myo*-inositol in guinea pig brain slices, but pimozide had no effect. It is unlikely, therefore, that this effect of chlorpromazine and haloperidol on phospholipid metabolism plays a major role in the neuroleptic actions that are common to these three drugs. There was no evidence that the increased synthesis of phosphatidylinositol involved a redirection of lipid synthetic pathways from the diacylglycerol pathway to the CDP-diacylglycerol pathway. Chlorpromazine and haloperidol appeared to block the effects of norepinephrine on phosphatidylinositol metabolism in cerebral cortex slices. The stimulation by these two drugs of [2-^{3}H]*myo*-inositol incorporation into phosphatidylinositol in corpus striatum slices was reduced in the presence of dopamine.

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