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ACTIONS OF PHENOTHIAZINE ANALOGUES ON DOPAMINE-SENSITIVE ADENYLATE CYCLASE IN NEURONAL AND GLIAL-ENRICHED FRACTIONS FROM RAT BRAIN

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Abstract—Dopamine (DA) at 10^{-4} M readily activated adenylate cyclase in homogenates of neuronal and glial-enriched fractions prepared from rat cerebral cortex, thalamus, striatum and the total homogenate from the striatum. Several derivatives of phenothiazines were tested for their ability to modify either the control component or the DA-sensitive receptor moiety of the enzyme. Dihydroxy analogues of chlorpromazine (CPZ), prochlorperazine, perphenazine, promazine and 7,8-dioxo-CPZ exhibited the most potent antagonism of either basal or DA-induced activation of the enzyme. In some cases at lowest concentrations the basal activity of adenylate cyclase was enhanced by these dihydroxy compounds. Parent compounds and corresponding monohydroxy metabolites of CPZ, prochlorperazine, perphenazine and fluphenazine were less potent toward antagonism of control enzyme preparations, but nevertheless exerted rather powerful antagonism at the DA-sensitive receptor site of adenylate cyclase. In this regard, 8-hydroxy derivatives were somewhat more potent than respective 7-hydroxy derivatives. Likewise, prochlorperazine and corresponding analogues were overall the most potent compounds. The 7-methoxy derivative of CPZ, along with thiothixene, thioridazine and haloperidol, was observed to exert a weaker antagonism of the DA-sensitive enzyme. Weakest inhibitory actions on either control or DA-sensitive sites of adenylate cyclase were seen with promazine, 2-OH- and 3-OH-promazine, clozapine, promethazine, CPZ-SO and 7,8-diMeO-CPZ. Phenothiazine, 3-OH-phenothiazine and 2-Cl-7,8-dioxo-phenothiazine were without effect. These findings suggest that molecular actions of pharmacologically active phenothiazines within the central nervous system are not totally reflected by the parent compounds, but may instead be additionally manifested by one or more metabolites.

Recent evidence suggests a central role for adenylate cyclase-cyclic adenosine 3′,5′-monophosphate (cyclic AMP) in synaptic transmission processes involving catecholamines [1, 2]. Moreover, the activation of adenylate cyclase by neurohumoral agents occurs in both neuronal and glial elements [3]. Robison *et al.* [4] have postulated that adenylate cyclase exists as an enzyme complex consisting of regulatory and catalytic components. The enzyme has been additionally shown to be an integral component of the adrenergic receptor. In this regard, several classes of psychotropic drugs which affect mood and behavior have been shown to affect central adenylate cyclase-cyclic AMP in a manner corresponding to their actions on central adrenergic and dopaminergic transmission mechanisms [5–15]. The pharmacologically active phenothiazines have been demonstrated to antagonize central adrenergic receptors [16] as well as the catalytic and receptor components of adenylate cyclase [5–13]. Recent preliminary investigations have shown the dopamine (DA)-sensitive receptor component of the enzyme to be especially susceptible to inhibition by phenothiazines [10–13]. It was the intent of the present study to investigate the effect of various antipsychotic agents on DA-sensitive adenylate cyclase in neuronal and glial-enriched fractions from different regions of the rat brain. Furthermore, we wished to correlate possible actions of phenothiazine derivatives on this enzyme system to the actions observed by the respective parent compounds.

MATERIALS AND METHODS

The authors are especially grateful to the following companies for generously supplying the analogues of phenothiazines used in the present study: chlorpromazine (CPZ), prochlorperazine, chlorpromazine sulfoxide and 3-OH-phenothiazine (Smith, Kline & French Laboratories); promethazine and promazine (Wyeth Laboratories); haloperidol (McNeill Laboratories); 3-OH-chlorpromazine (Rhone-Poulenc); fluphenazine and perphenazine (Schering Corp.); 8-OH-fluphenazine (Squibb Institute for Medical Research); thioridazine (Sandoz); and thiothixene (Pfizer). The Psychopharmacology Research Branch, N.I.M.H., provided the following derivatives: 7-OH-CPZ; 7,8-diOH-CPZ; 7,8-dioxo-CPZ; 8-OH-7-MeO-CPZ; 7-OH-8-MeO-CPZ; 7-MeO-CPZ; 7,8-diMeO-CPZ; 7-

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OH-prochlorperazine; 8-OH-prochlorperazine; 7,8-diOH-prochlorperazine; 7-OH-perphenazine; 8-OH-perphenazine; 7,8-diOH-perphenazine; 2-OH-promazine; 3-OH-promazine; 2,3-diOH-promazine; phenothiazine; 2-Cl-7,8-dioxo-phenothiazine; and clozapine.

Preparation of neuronal and glial-enriched fractions. The brain regions to be studied were rapidly removed from young adult male rats (Sprague–Dawley, Holtzman, weighing 60–80 g) and placed into a cold solution of 7·5% (w/v) polyvinyl pyrrolidone, 1% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo., U.S.A.) and 10 mM $CaCl_2$. The cellular fractions were isolated according to the procedure described by Sellinger *et al.* [17]. The tissue was minced and poured into a truncated disposable syringe and eased through successive passes of different pore sizes of nylon bolting cloth (333, 110 and 75 μm, three times each). The suspension was layered onto a discontinuous gradient consisting of 1·75 and 1·0 M sucrose and centrifuged at 20,000 rev/min in an SW 25·1 swinging bucket rotor for 30 min. Upon examination with phase microscopy, the pellet consisted of purified neuronal perikarya. The band containing the crude glial fraction was subjected to one additional density gradient centrifugation (1·65 M sucrose, 1·2 M sucrose and 30% (w/v) Ficoll). The glial-enriched fraction obtained at the 1·2 to 1·65 M interface after a 30-min centrifugation at 20,000 rev/min was contaminated with small neuronal nuclei, some broken cellular debris and capillaries. For the most part, however, this fraction consisted of intact astrocytes. The cells were suspended in cold glycylglycine buffer (2 mM + 1 mM $MgSO_4$, pH 7·4) and gently homogenized (four strokes) using a glass homogenizer with a Teflon pestle (clearance: 0·004 to 0·006 in.). An aliquot was removed for protein determination [18] and adenylate cyclase activity was measured in the remainder of the homogenate as previously described [3, 7]. The 250-μl incubation mixture consisted of the following constituents at final concentrations and volumes: (1) 50 μl of a buffer containing 3·3 mM theophylline and 40 mM Hepes buffer, pH 7·4, (2) 0·090 to 0·120 mg enzyme protein in 125 μl, (3) 50 μl of a mixture consisting of 2 mM ATP–3 mM $MgSO_4$ and (4) depending upon the conditions either 15 μl of a control solution or DA (10^{-4} M) and 10 μl of varying concentrations of phenothiazine compounds were included. All components of the reaction were mixed at 4° and the reaction was initiated by adding the Mg ATP mixture. Incubations were normally conducted for 12 min at 37° for the drug studies and for varying time periods in time course studies. Enzymatic reactions were terminated by boiling the samples for 5 min followed by cooling on ice and centrifugation at 1000 *g* for 10 min. An aliquot of the supernatant was removed (100 μl) and mixed with 25 μl of sodium acetate (0·25 M, pH 4·0). Cyclic AMP content in the samples was subsequently determined using the protein binding assay developed by Gilman [19]. Specific activity of adenylate cyclase was expressed as pmoles cyclic AMP formed/mg of protein/12 min. An ATP-regenerating system was omitted because several investigations have revealed that such a system is not necessary, provided that saturating amounts of ATP are present in the reaction mixture, the amount of enzyme protein is kept relatively small and the incubations are conducted for a short time period [3, 7, 10, 11, 13].

RESULTS

Effects of DA on adenylate cyclase Dopamine at 10^{-4} M readily activated adenylate cyclase in the total homogenate of the corpus striatum and in the neuronal fractions from the cerebral cortex, thalamus and striatum. In the glial-enriched fractions, DA was effective in only the cerebral cortex and the thalamus (Fig. 1). The lack of stimulatory effect by DA in the striatial glial fractions has been noted previously [3]. The per cent stimulation of adenylate cyclase by DA in the following order of decreasing potency was: thalamic neurons, 76 per cent; cerebral cortex neurons and glia, 43 and 42 per cent; striatial homogenate, 41 per cent; thalamic glia, 40 per cent; and striatial neurons, 27 per cent. The specific enzyme activities from the control adenylate cyclase preparations were highest in the cortical neurons > cortical glia > thalamic neurons > striatial glia > striatial neurons > thalamic glia > striatial homogenate (see Fig. 1). The incubation time selected for the enzyme preparations was 12 min because preliminary time

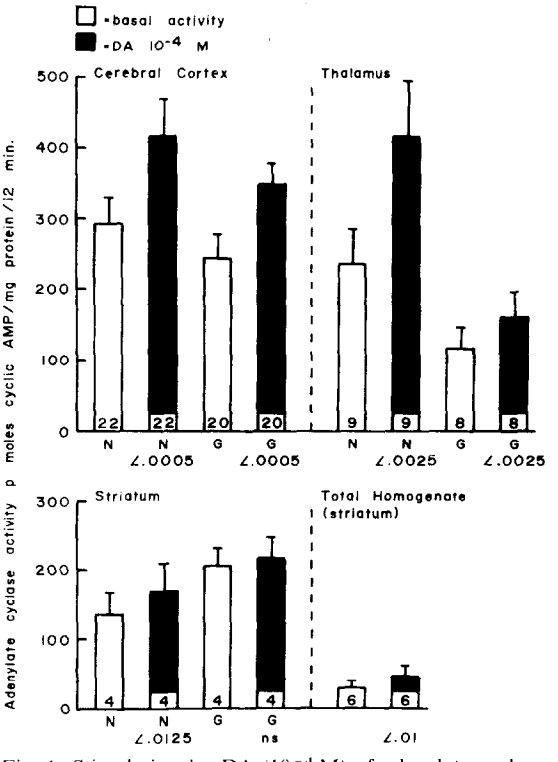

Fig. 1. Stimulation by DA (10^{-4} M) of adenylate cyclase in neuronal (N) and glial-enriched (G) fractions from rat cerebral cortex, thalamus, striatum and total homogenate of striatum. Mean enzyme activity ± S.E.M. is expressed as pmoles cyclic AMP produced/12 min/mg of sample protein. The number of individual determinations is denoted at the base of the individual bar graphs. Significant (Student's paired *t*-test) differences between respective control and DA-stimulated enzyme preparations are shown as P values immediately beneath the respective darkened bars; ns = not significant.

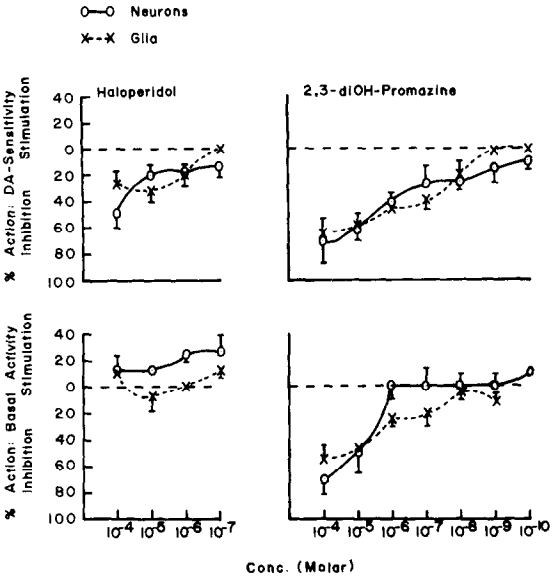

Fig. 2. Actions by phenothiazine analogues on either basal or DA-sensitive adenylate cyclase in disrupted neuronal or glial-enriched fractions of rat cerebral cortex. Enzymatic activity is expressed as pmoles cyclic AMP produced/12 min/mg of protein in the presence of absence of DA (10^{-4} M) along with various concentrations of phenothiazine analogues. The values in the figure represent the mean per cent inhibition or stimulation of adenylate cyclase activity ± S.E.M. of three to five determinations at specific drug concentrations. Basal and respective DA-stimulated enzyme activities are: 352 ± 96, 474 ± 102 (N = 4) for the neurons and 239 ± 14, 300 ± 18 (N = 3) for the glia.

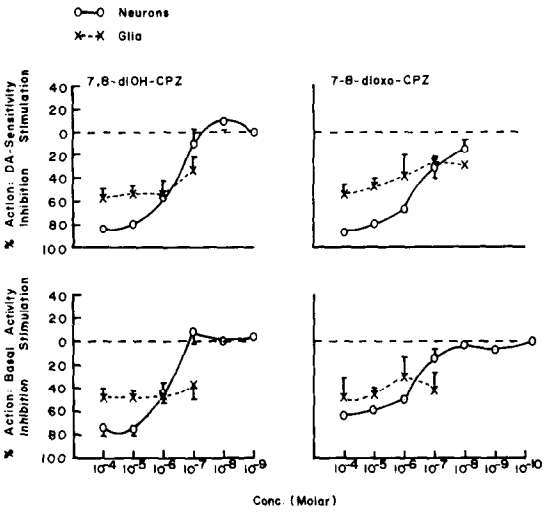

Fig. 3. Basal and respective DA-stimulated enzyme activities are: 315 ± 113, 405 ± 127 (N = 4) for the neurons and 163 ± 17, 218 ± 33 (N = 3) for the glia. For details, see legend to Fig. 2.

course studies revealed that under the conditions utilized in our assay system the maximum synthesis of cyclic AMP from ATP in the cellular fractions occurred from 12 to 20 min. The most detailed investigations were conducted using the neuronal and glial-enriched preparations from the cerebral cortex. The cellular yields from the thalamic glial fractions and the striatial preparations were extremely small. Therefore, we were only able to conduct limited studies with some of the CPZ compounds on these cells. The striatial homogenate was used to test a larger number of CPZ compounds in this brain area in order to verify observations by others [10, 12, 13] that this brain region was especially susceptible to inhibition by pharmacologically active phenothiazines.

Actions of phenothiazines in neuronal and glial-enriched fractions. The results of these studies are shown in Figs. 2–10 and Tables 1–3. As a general observation, the most potent antagonists toward either basal or DA-sensitive adenylate cyclase were the dihydroxylated derivatives of CPZ, prochlorperazine, perphenazine, promazine and 7,8-dioxo-CPZ (Figs. 2–4). These dihydroxylated compounds at lowest concentrations sometimes acted to elevate basal enzyme activity. However, the 2-Cl-7,8-dioxo analogue of the basic phenothiazine nucleus did not display anti-adenylate cyclase actions. Strong but lesser degrees of potency were seen with the following parent compounds and their respective monohydroxylated metabolites: CPZ, prochlorperazine, perphenazine, and fluphenazine (Table 1, Figs. 5–9). Likewise, thioridazine, haloperidol, thiothixene and 7-MeO-CPZ were effective (Table 1, Figs. 2 and 10). The agents demonstrating a lesser inhibitory action on adenylate cyclase were promazine and respective 2-OH and 3-OH analogues, promethazine, CPZ-SO and 7,8-diMeO-CPZ (Table 1, Fig. 10). No effects were seen with either phenothiazine or 3-OH-phenothiazine when compared to other compounds. The most potent actions on the enzyme systems were generally seen with prochlorperazine and respective derivatives. The individual groups of parent compounds and derivatives are discussed below in greater detail with regard to their actions on the neuronal and glial-enriched preparations.

Actions of parent compounds. With regard to an action on the control enzyme preparations, the highest concentrations (10^{-4} M) of CPZ, prochlorpera-

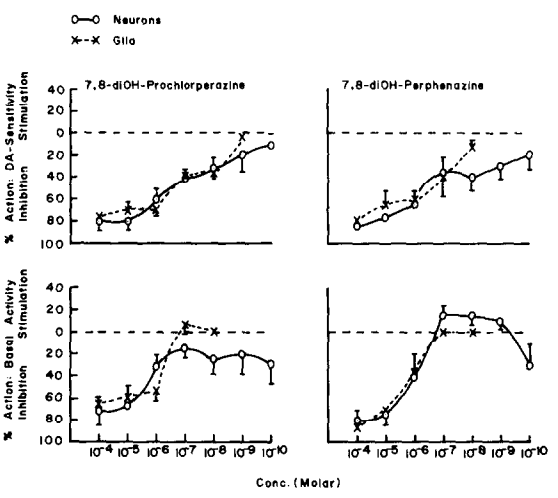

Fig. 4. Basal and respective DA-stimulated enzyme activities are: 326 ± 73, 486 ± 90 (N = 5) for the neurons and 236 ± 63, 339 ± 72 (N = 3) for the glia. For details, see legend to Fig. 2.

Table 1. Action of parent compounds of phenothiazines on either basal or DA-sensitive adenylate cyclase in neuronal and glial-enriched fractions from rat cerebral cortex*

Compound	Cell type	Per cent inhibition at specific drug concentrations (M)							
		Actions on basal activity				Actions on DA-stimulation			
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
CPZ	n	60 ± 3	13 ± 6	+13 ± 8	+25 ± 5	72 ± 4	37 ± 11	29 ± 10	−14 ± 13
	g	23 ± 11	17 ± 4	10 ± 5		45 ± 5	41 ± 6	36 ± 1	26 ± 8
Prochlorperazine	n	74 ± 8	37 ± 11	+16 ± 9	0	80 ± 6	56 ± 10	22 ± 12	29 ± 14
	g	72 ± 9	41 ± 10	12 ± 6	+13 ± 9	73 ± 5	46 ± 3	31 ± 6	32 ± 7
Perphenazine	n	0	0	+21 ± 10	+30 ± 15	46 ± 24	49 ± 13	43 ± 9	12 ± 9
	g	+19 ± 13	+27 ± 4	+6 ± 2	+23 ± 11	38 ± 15	49 ± 10	32 ± 12	19 ± 7
Promazine	n	0	0	+20 ± 11	+19 ± 7	29 ± 12	0	+8 ± 3	−8 ± 4
	g	16 ± 4	15 ± 6	+22 ± 11	+18 ± 11	21 ± 8	16 ± 6	10 ± 6	7 ± 4
Fluphenazine	n	29 ± 7	10 ± 7	+15 ± 10	+28 ± 12	54 ± 4	50 ± 7	23 ± 7	26 ± 9
	g	32 ± 12	0	+31 ± 15	+30 ± 18	56 ± 7	19 ± 11	28 ± 11	−13 ± 8
Promethazine	n	0	0	+21 ± 14	+23 ± 14	37 ± 15	22 ± 10	19 ± 12	17 ± 12
	g	0	0	+38 ± 20	+20 ± 10	21 ± 6	0	0	+12 ± 5
Thioridazine	n	68 ± 4	19 ± 10	+21 ± 7	+13 ± 6	75 ± 5	24 ± 6	23 ± 9	36 ± 12
	g	44 ± 8	+3 ± 2	+21 ± 13	0	54 ± 10	28 ± 13	14 ± 6	16 ± 8
Thiothixene	n	48 ± 13	10 ± 10	0	+20 ± 8	58 ± 17	26 ± 16	37 ± 13	41 ± 16
	g	34 ± 19	27 ± 9	14 ± 6	0	52 ± 17	0	0	0

* Disrupted cellular elements from neuronal (n) and glial-enriched (g) fractions were incubated in the absence or presence of DA (10^{-4} M) and various concentrations of phenothiazines. Enzymatic activity is expressed as pmoles cyclic AMP produced/12 min/mg of protein. The values in the table represent the mean per cent inhibition or stimulation (+) of either basal or DA-sensitive activity ± S.E.M. of three to four determinations. Basal enzyme activities were 266 ± 43 for the neurons and 218 ± 27 for the glia. Dopamine-stimulated enzyme activities were 385 ± 57 for the neurons and 319 ± 40 for the glia of 16 determinations.

zine, fluphenazine, thioridazine and thiothixene readily inhibited the enzyme. Prochlorperazine was the only compound effective at lower doses. Perphenazine, promazine, haloperidol and promethazine were without effects on basal adenylate cyclase activity and in some instances actually acted to enhance enzyme activity. Only minor differences were seen between the actions of these compounds on either the neuronal or the glial fractions (Tables 1–3, Fig. 2).

On the other hand, the DA-sensitive component of the enzyme was more susceptible to inhibition by the parent compounds. Again, prochlorperazine was seen to be the most potent agent (Table 1). Chlorpromazine, perphenazine, thioridazine and fluphenazine were effective inhibitors of the DA-sensitive enzyme at concentrations of 10^{-4} to 10^{-6} M (Tables 1 and 2). Thiothixene and haloperidol were equally effective but not to the degree of magnitude as the other compounds (Table 1, Fig. 2). Promazine and promethazine exhibited almost no antagonism of the enzyme (Table 1), while phenothiazine was completely ineffective.

Actions of dihydroxylated derivatives. These derivatives of CPZ, prochlorperazine, perphenazine, promazine and 7,8-dioxo-CPZ exerted the most profound antagonism of either basal or DA-stimulated adenylate cyclase from any cellular preparation (Figs. 2–4, Tables 2 and 3). With regard to inhibition of basal enzyme activity all compounds were active to 10^{-5}–10^{-6} M. The DA-sensitive moiety of adenylate cyclase

Table 2. Action by CPZ analogues on either basal or DA-sensitive adenylate cyclase in neuronal and glial-enriched fractions from rat thalamus*

Compound	Cell type	Per cent inhibition at specific drug concentrations (M)										
		Actions on basal activity						Actions on DA stimulation				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
CPZ	n	67 ± 5	42 ± 13	35 ± 18	36 ± 15			70 ± 9	53 ± 14	50 ± 19	29 ± 20	
	g	34 ± 15	23 ± 1	17 ± 6	7 ± 6			48 ± 13	38 ± 13	17 ± 10	27 ± 9	
7-OH-CPZ	n	52 ± 11	14 ± 7	0	0			56 ± 11	20 ± 8	0	0	
	g	38 ± 12	12 ± 8	0	0			49 ± 13	39 ± 11	19 ± 9	10 ± 6	
8-OH-CPZ	n	47 ± 14	0	+22 ± 12	+35 ± 4			74 ± 8	32 ± 11	0	0	
	g	27 ± 5	23 ± 11	12 ± 4	0			60 ± 10	32 ± 14	30 ± 15	10 ± 3	
3-OH-CPZ	n	+31 ± 11	+27 ± 6	+18 ± 5	+30 ± 9			38 ± 18	25 ± 15	15 ± 9	20 ± 16	
	g	15 ± 6	5 ± 4	13 ± 7	23 ± 8			47 ± 14	33 ± 12	44 ± 15	25 ± 9	
7,8-diOH-CPZ	n	74 ± 5	59 ± 4	+19 ± 6	+34 ± 6	+48 ± 4	+27 ± 2	85 ± 5	80 ± 7	66 ± 6	0	0
	g	59 ± 4	44 ± 9	16 ± 9	+35 ± 15	+16 ± 4		62 ± 2	63 ± 7	59 ± 3	25 ± 8	25 ± 15
7,8-dioxo-CPZ	n	75 ± 7	69 ± 10	55 ± 15	+13 ± 3	+33 ± 8	+10 ± 2	82 ± 11	79 ± 11	80 ± 1	34 ± 16	33 ± 5
	g	49 ± 10	37 ± 4	14 ± 6	+8 ± 3	+14 ± 6		69 ± 3	61 ± 17	51 ± 7	22 ± 7	0
CPZ-SO	n	0	0	0				26 ± 5	20 ± 3	9 ± 1		
	g	0	0	0				36 ± 4	31 ± 3	10 ± 8		

* Disrupted cellular elements from neuronal (n) and glial-enriched (g) fractions were incubated in the absence or presence of DA (10^{-4} M) and various concentrations of CPZ derivatives. Enzymatic activity is expressed as pmoles cyclic AMP produced/12 min/mg of protein. The values in the table represent the mean per cent inhibition or stimulation (+) of the basal or DA-sensitive enzyme ± S.E.M. of three to four determinations. Respective basal and DA-stimulated enzyme activities are shown in Fig. 1.

Table 3. Action by CPZ analogues on either basal or DA-sensitive adenylate cyclase in neuronal and glial-enriched fractions from rat striatum*

Compound	Cell type	Per cent inhibition at specific drug concentrations (M)									
		Actions on basal activity					Actions on DA stimulation				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
CPZ	n	40 ± 13 (3)	0	0	0		50 ± 9	28 ± 5	13 ± 5	0	
	g	36 ± 7 (3)	12 ± 7	6 ± 3							
7-OH-CPZ	n	43 ± 9 (3)	21 ± 7	0	0		55 ± 7	28 ± 9	21 ± 9		
	g	46 ± 2 (4)	26 ± 8	8 ± 4	0						
7,8-diOH-CPZ	n	53 ± 4 (2)	35 ± 4	42 ± 7	5 ± 3	+20 ± 6	65 ± 6	56 ± 7	58 ± 12	4 ± 2	25 ± 7
	g	75 ± 1 (2)	69 ± 4	50 ± 7	40 ± 6	+20 ± 6					
7,8-dioxo-CPZ	n	50 ± 4 (2)	30 ± 6	22 ± 7	+8 ± 4	+30 ± 8	94 ± 10	52 ± 12	55 ± 15	11 ± 5	+25 ± 9

* Disrupted cellular elements from neuronal (n) and glial-enriched (g) fractions were incubated in the absence or presence of DA (10^{-4} M) and various concentrations of CPZ derivatives. Enzymatic activity is expressed as pmoles cyclic AMP produced/12 min/mg of protein. The values in the table represent the mean per cent inhibition or stimulation (+) of the basal or DA-sensitive enzyme ± S.E.M. if three or four determinations were made and ± range if only two experiments were run. The numbers in parentheses denote the number of determinations for that particular drug. Respective basal and DA-stimulated enzyme activities are shown in Fig. 1.

was more susceptible to inhibition by these analogues. Moreover, 7,8-diOH-prochlorperazine and 7,8-diOH-perhenazine were active at concentrations as low as 10^{-8} to 10^{-9} M (Fig. 4). At times basal enzymatic activity was enhanced by low concentrations of 7,8-diOH-perphenazine (Fig. 4) and 7,8-diOH- and 7,8-dioxo-CPZ (Tables 2 and 3). The 2-Cl-7,8-dioxo derivative of phenothiazine was without any effects on either control or DA-stimulated enzyme activity.

Actions of 7-OH derivatives. The 7-OH metabolites of CPZ, prochlorperazine, perphenazine and 7-OH-8-MeO-CPZ exhibited weaker inhibitory actions toward either control or stimulated adenylate cyclase when compared to their respective parent compounds (Figs. 5 and 6, Table 2). The one exception was observed in the cellular preparations from the striatum (Table 3). At the lower drug concentrations, the basal enzyme activity was sometimes enhanced as evidenced with 7-OH-perphenazine (neurons) and 7-OH-8-MeO-CPZ (glia) (Figs. 5 and 6). In these studies on the control enzyme preparations, 7-OH-prochlorperazine was overall the most potent compound (Fig. 6).

The weakest antagonism of the DA-sensitive receptor component of adenylate cyclase occurred with 7-OH-8-MeO-CPZ (Fig. 5), while the ramainder of the compounds were generally effective from 10^{-4} to 10^{-6} M (Figs. 5 and 6, Tables 2 and 3).

Actions of 8-OH derivatives. These derivatives of CPZ, prochlorperazine, perphenazine and fluphenazine were more potent than the 7-OH analogues in their ability to inhibit either control or DA-induced

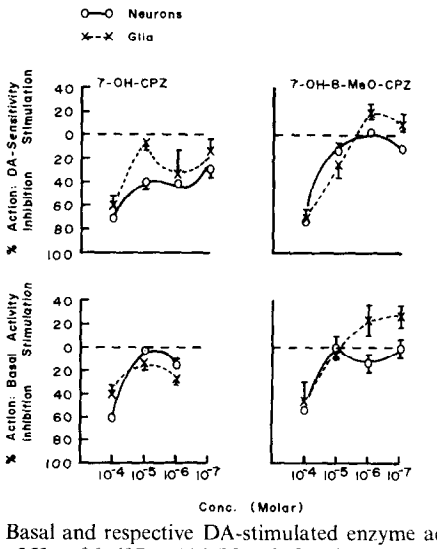

Fig. 5. Basal and respective DA-stimulated enzyme activities are: 353 ± 96, 487 ± 116 (N = 3) for the neurons and 214 ± 30, 306 ± 54 (N = 3) for the glia. For details, see legend to Fig. 2.

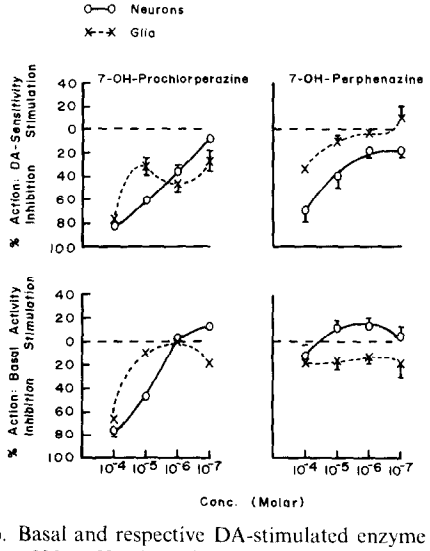

Fig. 6. Basal and respective DA-stimulated enzyme activities are: 290 ± 53, 436 ± 98 (N = 3) for the neurons and 255 ± 60, 342 ± 72 (N = 3) for the glia. For details, see legend to Fig. 2.

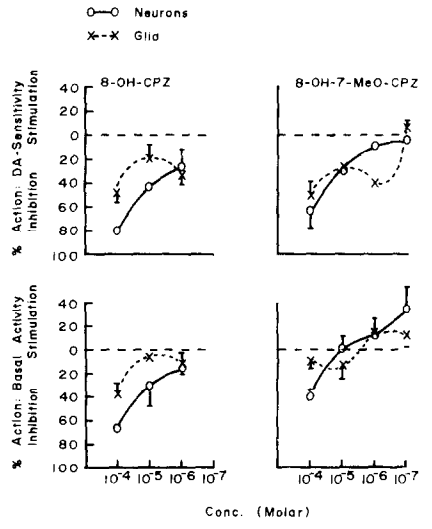

Fig. 7. Basal and respective DA-stimulated enzyme activities are: 200 ± 15, 312 ± 29 (N = 3) for the neurons and 190 ± 21, 273 ± 43 (N = 3) for the glia. For details, see legend to Fig. 2.

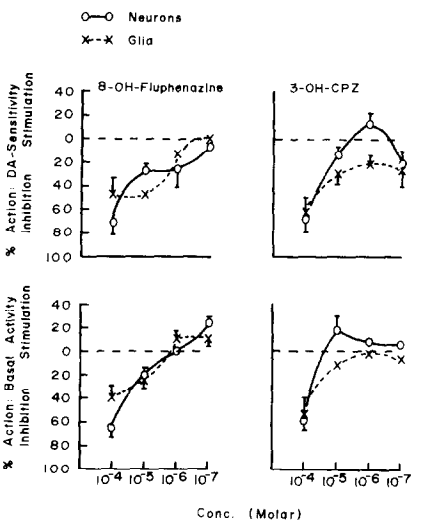

Fig. 9. Basal and respective DA-stimulated enzyme activities are: 262 ± 65, 544 ± 100 (N = 3) for the neurons and 193 ± 38, 375 ± 48 (N = 3) for the glia. For details, see legend to Fig. 2.

activation of the enzyme (Figs. 7–9, Table 2). With respect to an action on basal enzyme activity, 8-OH-prochlorperazine was the most potent compound (Fig. 8).

The DA-activated receptor locus of adenylate cyclase was diminished by all agents at 10^{-4} M (Figs. 7–9, Table 2). When the drugs were incubated at lower concentrations (10^{-6} M), an antagonism of DA-sensitive cyclase remained evident with 8-OH analogues of CPZ, prochlorperazine, fluphenazine and 8-OH-7-MeO-CPZ (cortical glia) (Figs. 7–9).

Actions of 2- and 3-OH derivatives. With regard to the control enzyme, 3-OH-CPZ inhibited to only a small degree adenylate cyclase in the thalamic and cortical preparations (Fig. 9, Table 2). The drug acted instead to enhance basal adenylate cyclase in the thalamic neurons (Table 2). The DA-sensitive sites of the enzyme were blocked to a greater extent in the glial preparations than in the corresponding neural enzymes (Fig. 9, Table 2). Neither 2-OH- nor 3-OH-promazine displayed any appreciable degrees of antagonism toward either basal or DA-sensitive components of the enzyme. Both compounds at 10^{-4} M inhibited the stimulation of adenylate cyclase by DA only 30 per cent. At lower concentrations these two analogues of promazine were relatively ineffective. 3-OH-phenothiazine was without any action on either cortical cellular preparation.

Actions of methoxylated and sulfoxide derivatives. 7-MeO-chlorpromazine exerted an antagonism of the control and DA-sensitive cortical enzyme that was especially evident in the neuronal cells (Fig. 10).

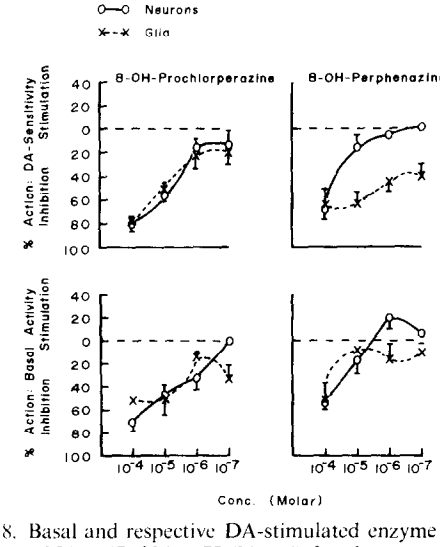

Fig. 8. Basal and respective DA-stimulated enzyme activities are: 254 ± 47, 384 ± 77 (N = 4) for the neurons and 236 ± 63, 339 ± 73 (N = 3) for the glia. For details, see legend to Fig. 2.

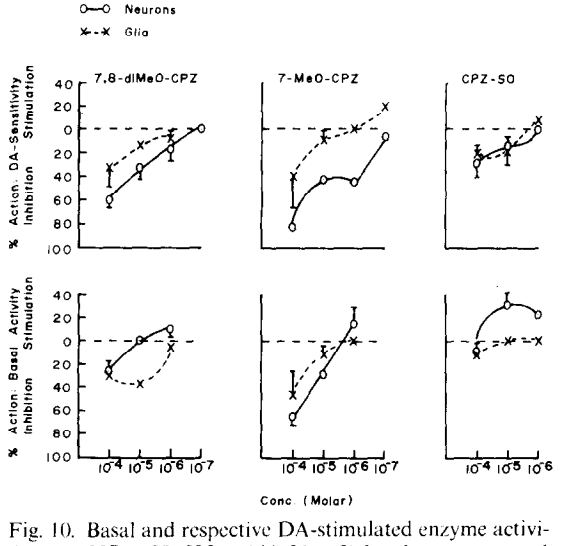

Fig. 10. Basal and respective DA-stimulated enzyme activities are: 287 ± 88, 505 ± 144 (N = 3) for the neurons and 332 ± 39, 456 ± 63 (N = 3) for the glia. For details, see legend to Fig. 2.

Table 4. Action by CPZ analogues on either basal or DA-sensitive adenylate cyclase in total homogenates of rat striatum*

		Per cent inhibition at specific drug concentrations (M)					
		Actions on basal activity			Actions on DA stimulation		
Compound	N	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}
CPZ	5	4 ± 2	0	0	32 ± 8	7 ± 3	0
7-OH-CPZ	4	0	0	+18 ± 6	19 ± 5	9 ± 4	0
8-OH-CPZ	4	20 ± 4	7 ± 3	0	32 ± 9	14 ± 6	0
3-OH-CPZ	4	32 ± 9	14 ± 6	0	54 ± 12	26 ± 8	0
7,8-diOH-CPZ	5	73 ± 7	27 ± 9	+9 ± 5	81 ± 5	38 ± 11	0
7,8-dioxo-CPZ	4	77 ± 3	27 ± 10	0	84 ± 3	57 ± 8	17 ± 11
CPZ-SO	4	0	0	0	0	0	0
Haloperidol	3	5 ± 1	0	0	28 ± 9	26 ± 4	11 ± 5
Thiothixene	3	13 ± 5	0	0	37 ± 11	33 ± 13	20 ± 7
Thioridazine	3	11 ± 2	0	0	41 ± 9	29 ± 10	16 ± 8
Clozapine	3	0	0	0	27 ± 4	17 ± 9	11 ± 6

* Samples were lightly homogenized and aliquots containing 0·333 μg protein were incubated in the absence or presence of DA (10^{-4} M) and various concentrations of CPZ derivatives. Enzymatic activity is expressed as pmoles cyclic AMP produced/12 min/mg of protein. The values in the table represent the mean per cent inhibition or stimulation (+) of the basal or DA-sensitive enzyme ± S.E.M. N = number of determinations. Respective basal and DA-stimulated enzyme activities are shown in Fig. 1.

Chlorpromazine-SO and 7,8-diMeO-CPZ were seen to be relatively ineffective toward either basal or stimulated adenylate cyclase in the cortical fractions, but CPZ-SO did exhibit inhibition in the thalamus (Fig. 10, Table2).

Actions of CPZ and derivatives, haloperidol, thiothixene, thioridazine and clozapine on DA-sensitive adenylate cyclase in the total homogenate of the striatum. The results of these studies are shown in Table 4. Basal activity of the enzyme was considerably lower than in the more purified neuronal and glial-enriched fractions. The activation of the receptor moiety of adenylate cyclase by DA (10^{-4} M) was, however, to approximately the same magnitude (41 per cent) as observed with the cellular preparations (Fig. 1). Inhibition of either basal or stimulated enzyme by the CPZ analogues was evident at 10^{-4} M but did not occur to the same extent as seen in the isolated cells. The diOH and dioxo derivatives of CPZ were again the most potent compounds and effectively inhibited the enzyme components at 10^{-4} and 10^{-5} M. Chlorpromazine and respective 7-OH and 8-OH metabolites were ineffective on control enzyme preparations and exhibited antagonism of the DA-sensitive site at only 10^{-4} M. The 3-OH analogue was considerably more effective, while CPZ-SO was without any actions. Haloperidol, thiothixene, thioridazine and clozapine have little actions at the control site of adenylate cyclase. Haloperidol, thiothixene and thioridazine equally displayed an inhibition of the DA-sensitive enzyme at concentrations from 10^{-4} to 10^{-6} M. Clozapine, however, exerted a somewhat weaker antagonism of the DA-sensitive enzyme.

DISCUSSION

Adenylate cyclase is postulated to be an integral component of adrenergic receptors [4]. In the rat brain, the catecholamines (norepinephrine, epinephrine and isoproterenol) readily stimulate this enzyme [8, 9, 15, 20]. Investigations concerning the activation of adenylate cyclase by DA have been somewhat controversial. In the earlier studies utilizing incubated tissue slice or other intact cellular preparations, the investigators were unable to demonstrate the presence of a DA-sensitive receptor component for adenylate cyclase [15, 21–24]. In subsequent studies, the presence of a DA-sensitive enzyme was demonstrated in homogenates of bovine superior cervical ganglion [1] and several areas of the rat brain including the striatum [10, 12, 13, 25], cerebral cortex [11], nucleus acumbens [12], olfactory tubercle [12], retina [26] and neuronal and glial-enriched fractions from different brain regions [3]. Recently, J. P. Perkins (personal communication) and Prasad *et al.* [27] reported that DA would stimulate the accumulation of cyclic AMP in tissue slices and neuroblastoma cells, provided that adequate concentrations of phosphodiesterase inhibitors were present. Moreover, recent findings revealed that DA would stimulate cyclic AMP production in incubated striatial and hypothalamic tissues *in vitro* [28, 29]. Our present experiments with broken cellular preparations confirm the presence of the DA-sensitive adenylate cyclase reported in these previous investigations [1, 10, 13, 25–29]. In our investigations we were only able to demonstrate an approximate 41 per cent increase in enzyme activity when DA was added to the cellular homogenates. These results are somewhat less than the more than 2-fold stimulation of adenylate cyclase in several central preparations by DA as reported by previous investigators [1, 10–12, 28]. At present we are unable to explain this discrepancy except for the different methodologies in use among the various laboratories.

Studies using histochemical fluorescent and biochemical techniques demonstrated the presence of dopaminergic nerve endings in the cerebral cortex, striatum and limbic system [30–32]. The presence of dopaminergic endings in the thalamus has not been determined; however, we found that DA readily activated adenylate cyclase in this region. Whether the receptors for DA and norepinephrine are separate in these brain areas is not known. However, recent investigations with the DA-sensitive adenylate cyclase in the bovine superior cervical ganglion and the rat

caudate nucleus argue in favor of a separate DA-sensitive receptor [1, 10].

Robison *et al.* [4] postulated that adenylate cyclase existed as an enzyme complex consisting of functional components. The receptor moiety is thought to face the exterior of the cell and respond to only specific hormones. The catalytic site of the enzyme is responsible for the synthesis of cyclic AMP from ATP. Moreover, the catecholamine hypothesis suggests that hypersensitive receptors or excess concentrations of DA and norepinephrine may be involved in the pathophysiology of schizophrenia [33]. In this regard, the therapeutic effects of pharmacologically active phenothiazines have been attributed to their ability to block central adrenergic and dopaminergic receptors [16, 34]. The dopaminergic receptors are extremely sensitive to the action of phenothiazines, and drug-induced Parkinsonism is a prominent manifestation of DA receptor blockade [35]. A series of recent investigations with psychotropic drugs has suggested an involvement of adenylate cyclase activity in changes in mood and behavior [5 15]. In many of these investigations, the pharmacologically active phenothiazines were found to exert powerful inhibitory actions at either the catalytic or receptor or both sites of the enzyme [5–13, 36–38]. Perhaps some of the therapeutic qualities of the psychoactive phenothiazines are manifested at the level of central adenylate cyclase within particular regions of the brain.

The hydroxylated metabolites or derivatives of phenothiazines have been demonstrated to have a variety of pharmacological properties, at times minicking the actions of their parent compounds. These properties include actions on various behavioral and physiological situations in laboratory animals [39–41] and influences on a host of central enzymes that include inhibition of ATPases [42], protein kinase and phosphodiesterase inhibition,* an evoked Ca^{2+} efflux and inhibition of oxygen uptake in mitochondria [43], binding to cellular membranes [44], and inhibition of the catalytic and receptor components of central adenylate cyclase [6 8, 13]. It is noteworthy that the dihydroxylated analogues are the most potent in almost all of these experimental situations. With regard to adenylate cyclase actions, the dihydroxyphenothiazines do not influence to any appreciable extent the norepinephrine-induced accumulation of cyclic AMP in intact cells [8]. Furthermore, these compounds at specific concentrations actually tend to enhance basal activity of the cyclic nucleotide as well as adenylate cyclase as observed here and elsewhere [7, 8].

In the present experiments, the 8-OH analogues of CPZ, prochlorperazine, perphenazine and fluphenazine were somewhat more potent than respective 7-OH derivatives in their ability to modify either control or DA-incuded activation of the enzyme. This is contrasted by previous work on either the catalytic or norepinephrine-sensitive components of the enzyme in which the 7-OH compounds were more active [6 8]. Likewise, additional observations have denoted that the 7-OH metabolites are more active in many pharmacological and behavioral investigations [39, 41]. Our data do show that the 8-OH compounds are more potent toward dopamine-sensitive adenylate cyclase receptors and hopefully future studies may resolve this conflict.

Sulfoxide and methoxy analogues of phenothiazines are generally less potent than parent compounds in actions on adenylate cyclase [6–9, 13]. In our investigations, the 7-methoxy analogue of CPZ, though not a reported metabolite, was a rather potent inhibitor of either basal or dopamine-sensitive sites of the enzyme in the cerebral cortex.

Thioridazine, haloperidol and thiothixene have been recognized to possess stronger blocking actions at central dopaminergic receptors when compared to central adrenergic receptors [34, 45–48]. In addition, clozapine, a relatively recent antipsychotic drug, is reported to be a weaker antagonist of central DA receptors (for discussion) [49]. In the present investigation, the DA-sensitive adenylate cyclase was antagonized by these compounds and these results support recent findings [10–12]. Earlier observations revealed that haloperidol did not influence either the basal activity or the catalytic site and acted to modify to a limited extent the norepinephrine-sensitive component of the enzyme [5, 7, 8, 10]. Furthermore, thiothixene was shown to inhibit the norepinephrine-induced accumulation of cyclic AMP in whole cell preparations [8]. Promethazine, a phenothiazine devoid of antipsychotic activity, was observed in these and previous investigations to have limited actions at the DA- and norepinephrine-sensitive components as well as the catalytic site of the enzyme [7–10, 12].

In conclusion, it is highly probable that metabolites of the pharmacologically active phenothiazines additionally contribute toward the overall antidopaminergic and anti-adrenergic activities of the parent compounds. To what degree these metabolites are partially responsible for either the therapeutic antipsychotic effects and/or the adverse actions of the parent compounds remains to be determined.

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* P. Greengard and G. Petzold, personal communication.

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