Biochemical Pharmacology, Vol. 25, pp. 537–541. Pergamon Press, 1976. Printed in Great Britain.

COMPARISON OF THE INHIBITORY EFFECTS OF NEUROLEPTIC DRUGS ON ADENYLATE CYCLASE IN RAT TISSUES STIMULATED BY DOPAMINE, NORADRENALINE AND GLUCAGON

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(*Received* 16 *June* 1975; *accepted* 20 *August* 1975)

Abstract—The effect of neuroleptic drugs of various chemical classes on adenylate cyclase from a variety of rat tissues has been investigated. These were the dopamine-stimulated adenylate cyclase in rat striatal homogenates, the noradrenaline-stimulated adenylate cyclase in adipocyte membrane fragments and the glucagon-stimulated adenylate cyclase in liver membranes. The stimulatory effects of dopamine on rat striatal adenylate cyclase were apparent within a minute of the start of incubations and over a wide range of temperatures. Moreover the stimulation could be rapidly inhibited by several classes of neuroleptic agent. The inhibitory effect of neuroleptics of all classes was completely reversed by high concentrations of dopamine and this reversal took place rapidly at 30°. Neuroleptics were also able to inhibit the stimulatory effects of noradrenaline on adipocyte adenylate cyclase at concentrations where the basal enzyme activity was not affected. However the effects of drugs in this system occurred at much higher concentrations than in the dopamine-stimulated system. In addition drug potencies as inhibitors of noradrenaline did not correlate with neuroleptic activity, α and β-flupenthixol for example were equipotent as noradrenaline antagonists. Neuroleptics were able to inhibit the stimulation of liver membrane adenylate cyclase by glucagon. The potencies of drugs as inhibitors in this system again did not correlate with neuroleptic potencies. Higher concentrations of glucagon were not able to reverse the inhibition produced by neuroleptics. It is concluded that the effects of neuroleptic drugs on dopamine-stimulated adenylate cyclase correlate most closely with their clinical neuroleptic potencies.

Consideration of the possible molecular basis of neuroleptic drug action has led several investigators to examine the effects of these agents on hormone sensitive adenylate cyclase systems. It has been shown that the stimulatory effects of various hormones on adenylate cyclases or cyclic AMP accumulation in whole cells can be inhibited by neuroleptics. Such inhibition has been described for noradrenaline [1, 2], dopamine [3, 4], ACTH [5], thyrotropin [1], parathyroid horhormone [1], glucagon [1], prostaglandin E_1 [1], histamine [5] and fluoride stimulated enzymes [2]. The tissues used have included thyroid [1], brain [2, 3, 4], adrenal [5], kidney cortex [1] and liver [1]. It has been suggested that the inhibition seen is related to the clinical action of the drugs. However, in most cases the action of a few drugs, usually from only one chemical class (i.e. phenothiazines) has been examined. Moreover, the mechanism of the drug/cyclase interaction has not in general been examined. It is, therefore, not possible to say whether these effects correlate well with neuroleptic potency in most studies. It is also not possible to say whether many of the above effects represent an interaction of the drug with the hormone receptor or with another component of the adenylate cyclase complex.

Because of the large volume of clinical and neurochemical data suggesting that neuroleptic drugs act as antagonists at central dopamine receptors [6, 7], we and others have examined the effects of neuroleptic drugs on the dopamine-sensitive adenylate cyclase present in brain areas rich in dopaminergic synapses. Neuroleptic drugs of all chemical types will inhibit the stimulatory effects of dopamine on adenylate cyclase and in most cases this inhibition correlates well with neuroleptic potency [3, 4]. However, there are certain exceptions and in these cases alternative modes of action have been suggested.

In the present investigation the interaction of neuroleptic drugs with the dopamine stimulated adenylate cyclase and two other hormone sensitive adenylate cyclases has been examined in order to elucidate the mechanism of this interaction, and determine which effects may be the most relevant in relation to the clinical action of these drugs.

MATERIALS AND METHODS

Dopamine-sensitive adenylate cyclase. Dopamine-sensitive adenylate cyclase in rat brain striatal homogenates was assayed as previously described [4].

Noradrenaline-sensitive adenylate cyclase. Noradrenaline-stimulated adenylate cyclase in isolated fat cells was assayed as follows. Isolated adipocytes were prepared from the epididymal fat pads of rats by the method of Rodbell [8]. After washing with buffer containing dialyzed albumin, the cells were homogenized at 4°C in 50 mm Tris–HCl pH 7·6 and centrifuged

at 100,000 *g* for 20 min. Enzyme assays were performed on the resulting pellet after resuspension in 50 mm Tris HCl pH 7·7. The assay system (final volume 100 μl) consisted of 50 mm Tris HCl pH 7·7, 6 mM $MgCl_2$, 1 mM isobutylmethylxanthine, 0·5 mg/ml creatine phosphokinase, 10 mM creatine phosphate, 3·2 mM ATP, 0·1% bovine serum albumin and approximately 50 μg adipocyte membrane protein plus drugs as indicated. Incubations were initiated by the addition of ATP and carried out for 10 min in a shaking water bath at 30°. The reaction was terminated by placing tubes in a boiling water bath for 2·5 min. Each tube was centrifuged to remove denatured protein, and the supernatant was assayed for cyclic AMP content by the method of Brown *et al.* Protein was determined by the method of Lowry *et al.* [10].

Glucagon-stimulated adenylate cyclase. Partially purified liver plasma membranes were prepared by the method of Purkis *et al.* [11], which is a modification of the method of Song *et al.* [12]. Once prepared, the membranes were stored at −70° in small aliquots. Glucagon-stimulated adenylate cyclase was assayed under the following conditions, final incubation volume 50 μl, 50 mM Tris HCl buffer pH 7·7, 20 mM creatine phosphate, 1 mg/ml creatine phosphokinase, 1 mM EDTA, 1 mM isobutylmethylxanthine, 3·2 mM ATP, 5 mM $MgSO_4$ and approximately 25 μg of membrane protein. The reaction was initiated by addition of ATP, and incubations carried out at 30° for 10 min. The reaction was terminated and cyclic AMP content determined as described above.

Materials. Dopamine, L-noradrenaline and glucagon were purchased from SIGMA. Creatine kinase, creatine phosphate and ATP were from Boehringer. Isobutylmethylxanthine was from Aldrich. Pimozide was given by Janssen Pharmaceutica, thioridazine and clozapine by Sandoz, α- and β-flupenthixol by Lundbeck Ltd. and other drugs by May and Baker Ltd.

RESULTS

Dopamine-sensitive adenylate cyclase. It has been shown previously that neuroleptic drugs of varying chemical structures inhibit the stimulation of striatal adenylate cyclase by dopamine. The potencies of the butyrophenone type of neuroleptic do not correlate well, however, with their '*in vitro*' potencies. The dynamics of interaction of various classes of drug with this system was, therefore, investigated. Dopamine acted very rapidly after addition to striatal homogenates, and stimulation of cAMP formation was seen at one min, the shortest time point examined. Both α-flupenthixol and pimozide inhibited the effects of dopamine within one minute of addition to the incubation (Fig. 1). In another experiment in which α-clopenthixol and haloperidol were added initially to the incubations it was found that a high concentration of dopamine (10^{-3} M) rapidly reversed the effects of these drugs after its addition to the incubation (Fig. 2). It, therefore, seems that both tricyclic and butyrophenone type neuroleptic agents interact in a rapidly reversible manner with the dopamine-sensitive adenylate cyclase. The effect of temperature on the inhibitory potencies of different types of neuroleptics was also examined. Fig. 3 shows the effect of temperature on stimulation of striatal adenylate cyclase by dopamine. It can be seen that dopamine was effective in stimulating adenylate cyclase over a wide range of temperatures. The blockade of dopamine (10^{-4} M) stimulation of adenylate cyclase was examined at 30° and 37°. At both temperatures the inhibitory profiles of chlorpromazine and pimozide were the same.

The effect of various neuroleptic agents on the dose-response curve for stimulation of adenylate cyclase by dopamine was also examined. When tested at concentrations close to their IC_{50} values the inhibitory effects of all neuroleptics were reversed by high concentrations of dopamine. Fig. 4 shows the effect of pimozide on the dopamine dose-response curve.

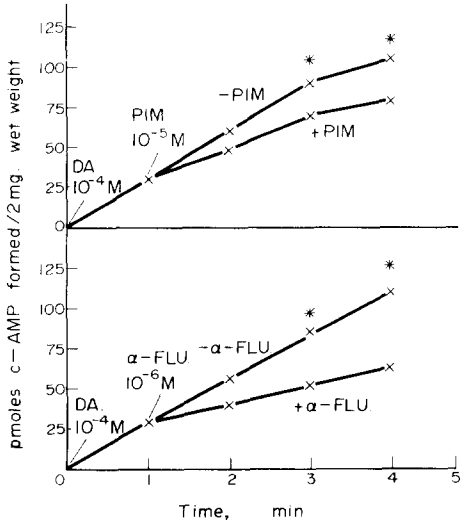

Fig. 1. Time course of the inhibitory effect of pimozide (PIM) and α-flupenthixol (α-FLU) on stimulation of striatal adenylate cyclase by dopamine 10^{-4} M. Points are means of 3 separate incubations.
* $P < 0.05$.

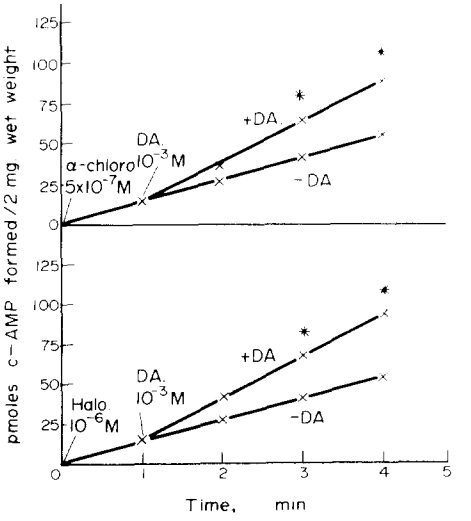

Fig. 2. Time course of the reversal of inhibition of striatal adenylate cyclase by α-chlorprothixene (α-CHLORO) and haloperidol (HALO) by dopamine 10^{-3} M. Points are means of 3 separate incubations.
* $P < 0.05$.

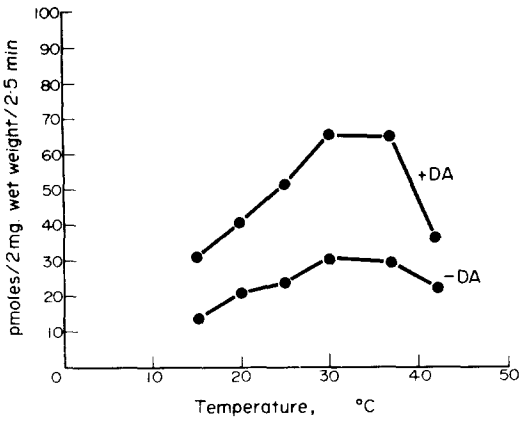

Fig. 3. Effect of temperature on stimulation of striatal adenylate cyclase by dopamine 10^{-4} M. Points are means of 3 separate incubations.

The drug caused a parallel shift of the dose-response curve to the right, and this is compatible with a competitive mode of inhibition. Similar effects were seen with thioridazine, pimozide, haloperidol, chlorpromazine and spiroperidol. The chemical classes of these drugs are shown in Table 1.

Noradrenaline-stimulated adenylate cyclase. The adenylate cyclase in membrane fragments from isolated adipocytes was stimulated by noradrenaline and fluoride as previously reported [13, 14]. Increasing concentrations of various neuroleptic drugs inhibited the effects of a maximally stimulating concentration of noradrenaline (10^{-4} N) (Fig. 5). Up to concentrations of 5×10^{-4} M no drug inhibited basal or fluoride-stimulated adenylate cyclase activity. At 10^{-3} M, however, chlorpromazine and α- and β-flupenthiol inhibited basal and fluoride-stimulated activity as well as noradrenaline-stimulated activity. It should be noted that considerably higher concentrations of drugs were required to inhibit the effects of a maximally stimulating concentration of noradrenaline than a maximally stimulating concentration of dopamine in the striatal system. Moreover, α- and β-flupenthixol had similar potencies as antagonists of the noradrenaline response, and α-flupenthixol was less potent than chlorpromazine. This does not parallel the neuroleptic potencies of these agents, since α-flupenthixol is considerably more potent than chlorpromazine as a neuroleptic, and β-flupenthixol is virtually devoid of neuroleptic activity. It was found that the inhibition produced by chlorpromazine (2×10^{-4} M) could be completely reversed by higher concentrations of noradrenaline indicating that this drug may act at least in part as a competitive antagonist with noradrenaline for the β-adrenoceptor, although the potency of chlorpromazine in this respect is at least two orders of magnitude lower than for the dopamine receptor [4].

Glucagon-stimulated adenylate cyclase. As has been shown previously, adenylate cyclase activity in liver membranes was stimulated by glucagon [11], noradrenaline [15] and fluoride [15]. It was found that high concentrations of chlorpromazine could inhibit the stimulation produced by a maximally stimulating concentration of glucagon (0·1 μg/ml). The two isomers of flupenthixol were also able to inhibit the effects of glucagon, and their inhibitory effects were both similar in potency to those shown by chlorpromazine (data not shown) (Fig. 6). This again does not parallel the neuroleptic potencies of these drugs. At concentrations up to 5×10^{-4} M no drug inhibited basal or fluoride stimulated activity, but at 10^{-3} M the drugs tested did inhibit both of these activities. In addition, the inhibition produced by 5×10^{-4} M chlorpromazine could not be reversed by higher concentration of glucagon (Fig. 7).

DISCUSSION

Because of the large literature describing the interaction of neuroleptic drugs with adenylate cyclase systems it seems important to establish which of these

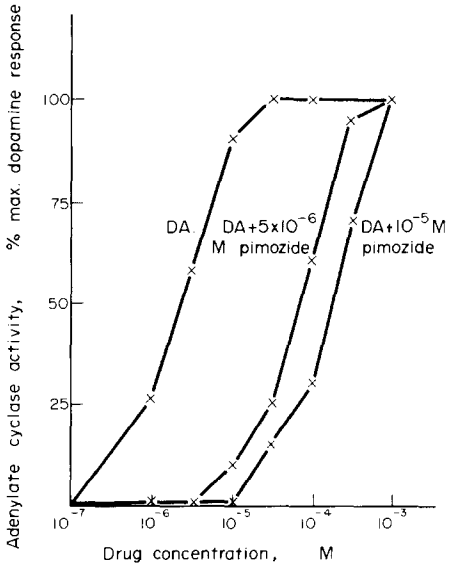

Fig. 4. Effect of pimozide 5×10^{-6} and 10^{-5} M on stimulation of striatal adenylate cyclase by dopamine. Mean basal activity was 28·3 ± 3·5 pmol cAMP/2mg wet weight/2·5 min. In the absence of pimozide and the presence of 10^{-4} M dopamine (maximal activiation) activity was 58·0 ± 3·7 pmol cAMP/2mg wet weight/2·5 min. Points are means of three or more separate incubations.

Table 1. Effects of neuroleptic drugs on hormone stimulated adenylate cyclases

Drug	Class	*IC_{50} Striatum (M)	IC_{50} Adipocyte (M)	IC_{50} Liver (M)
Chlorpromazine	Phenothiazine	1×10^{-6}	2×10^{-4}	5×10^{-4}
α-Flupenthixol	Thioxanthene	$2{\cdot}2 \times 10^{-8}$	$3{\cdot}5 \times 10^{-4}$	5×10^{-4}
β-Flupenthixol	Thioxanthene	$> 10^{-4}$	$3{\cdot}5 \times 10^{-4}$	5×10^{-4}
Haloperidol	Butyrophenone	2×10^{-6}	4×10^{-5}	$> 10^{-3}$
Promazine	Phenothiazine	6×10^{-5}	—	$> 10^{-3}$

* Some of this data is taken from Miller *et al* [4] and from Clement-Cormier *et al* [3].

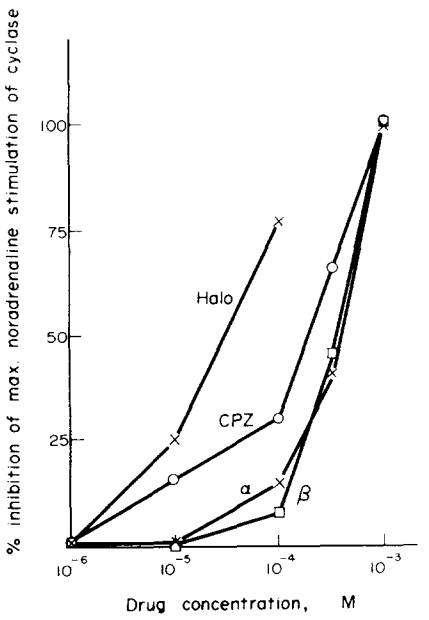

Fig. 5. Effect of neuroleptic drugs on maximal stimulation of adipocyte membrane adenylate cyclase by noradrenaline 10^{-4} M. Drugs used were haloperidol (HALO), chlorpromazine (CPZ) and α- and β-flupenthixol (α) and (β). Basal enzyme activity was 0·57 ± 0·03 nmol cAMP/mg protein/10 min. Activity in the presence of 10^{-4} M noradrenaline was 2·81 ± 0·11 nmol cAMP/mg protein/10 min. Points are means of five separate incubations.

effects if any are related to the clinical effects produced by these agents. The present study compares the effects of some neuroleptic drugs on three of these systems. It has been reported previously that neuroleptic drugs potently inhibit the stimulation by dopamine of adenylate cyclase activity in homogenates of various dopamine-rich regions of the brain [3, 4, 16]. In the case of phenothiazines, thioxanthenes and dibenzodiazepines the inhibitory potencies of drugs in this system correlate well with clinical effects. However, butyrophenones such as haloperidol and pimozide are much more potent *in vitro* than in the adenylate cyclase system, although they do have some effects in the latter [3, 4]. It has been suggested that such agents may have a predominantly pre-synaptic action as inhibitors of dopamine release [17]. The experiments reported here, therefore, examined the possibility that the mode of inhibition of the effect of dopamine by butyrophenones may differ in some way from other types of neuroleptics. It was found, however, that neuroleptics of all types of structure tested inhibited the effects of dopamine in a competitive fashion. It was also found that drugs of different structures acted rapidly on incubation with striatal homogenates, and that this inhibition could also be reversed rapidly by high concentrations of dopamine. Moreover, performing the assays at a higher temperature (37°) did not alter the inhibitory effects of drugs tested. It, therefore, appears that neuroleptic drugs of differing structures interact with dopamine in this system in the same manner. That the dopamine-stimulated adenylate cyclase is situated postsynaptically may be inferred since denervation of the nigrostriatal pathway electrolytically or with 6-hydroxydopamine does not decrease, and may in fact increase, the activity of this enzyme [18]. It has also been found recently that pimozide will antagonize the behavioural effects of apomorphine in animals with 6-hydroxy-dopamine induced lesions [19]. Such observations indicate that butyrophenone type agents have a predominantly postsynaptic mode of action. The increased potency of the butyrophenones *in vitro*

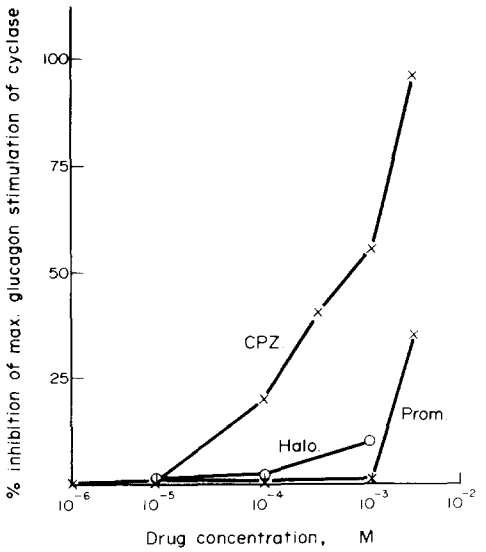

Fig. 6. Effect of neuroleptic drugs on maximal stimulation of liver membrane adenylate cyclase by glucagon 0·1 μg/ml. Drugs used were chlorpromazine (CPZ) haloperidol (HALO) and promazine (PROM). Basal enzyme activity was 0·19 nmol cAMP/mg protein/10 min. In the presence of 0·1 μg/ml glucagon (maximal stimulation) activity was 1·33 nmol cAMP/mg protein/10 min. Points are means of five separate incubations.

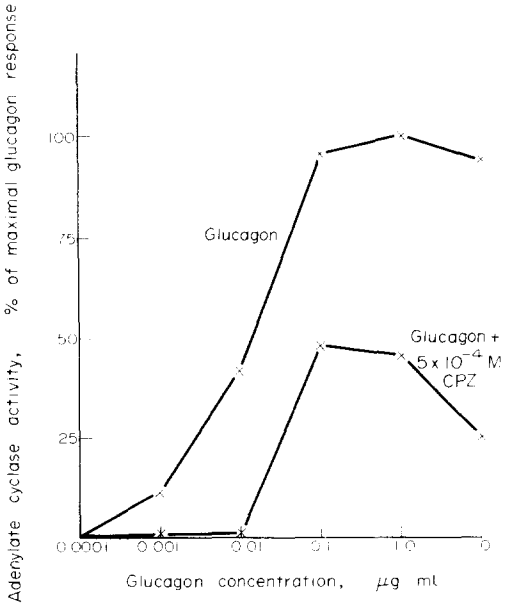

Fig. 7. Effect of chlorpromazine (CPZ) 5×10^{-4} M on stimulation of liver membrane adenylate cyclase by glucagon. Basal enzyme activity was 0·26 nmol cAMP/mg protein/10 min. In the presence of 0·1 μg/ml glucagon activity was 1·98 nmol cAMP/mg protein/10 min. Points are means of five separate incubations.

may, therefore, be due to other pharmacokinetic factors, as previously suggested [20].

As observed by other authors we also found that neuroleptic drugs will interact with noradrenaline and glucagon stimulated adenylate cyclases [1, 2]. It has been reported that the effects of noradrenaline applied microiontophoretically to the Purkinje neurones of the cerebellum may be inhibited by neuroleptic agents such as α-flupenthixol and fluphenazine [21]. This receptor has been previously shown to have some of the characteristics of a β-adrenoceptor [22]. The experiments reported here show that these drugs will inhibit the stimulating effects of noradrenaline on a classical β-adrenoceptor linked adenylate cyclase in adipocytes. However, certain features of this inhibition should be noted. Firstly although these effects occur at concentrations at which basal enzyme activity was not affected, the drug concentration needed are at least 100 times higher than those required to inhibit the stimulating effects of dopamine on striatal adenylate cyclase. Secondly, the system does not distinguish between the active and inactive α- and β-isomers of flupenthixol, whereas the dopamine-stimulated system does. Thirdly, chlorpromazine in the adipocyte system is more potent than α-flupenthixol, Which again does not parallel behavioural data which show that α-flupenthixol is considerably more potent [4]. In the dopamine-stimulated system α-flupenthixol is considerably more potent than chlorpromazine. In general, therefore, the inhibitory effects of these neuroleptics on β-adrenoceptors do not parallel their clinical neuroleptic activity. However, chlorpromazine may have some affinity for the β-adrenoceptors since high concentrations of noradrenaline will reverse the inhibition produced by chlorpromazine. As the precise pharmacological classification of noradrenaline receptors in the brain is not known, it may be that they are more sensitive to blockade by neuroleptics than those in the periphery.

The effects of neuroleptics on adenylate cyclase stimulated by polypeptide hormones are harder to assess. The present results indicate that as with the noradrenaline stimulated system the inhibitory effects of neuroleptics in the glucagon-sensitive system do not correlate with clinical activity, and require very high concentrations. Moreover, the effects of chlorpromazine could not be overcome by high concentrations of glucagon. It may be that high concentrations of neuroleptics interact with the membrane receptor system to inhibit the binding of the hormone. Another possibility is that the drugs interact with membrane components such as phospholipids which are thought to be important in 'coupling' hormone receptors to adenylate cyclase [23, 24]. At any rate at the concentrations at which neuroleptics produce such effects, they also affect the enzymatic functions of other 'integral proteins', such as the Na^+/K^+ATP-ase [25].

Of all interactions so far reported of neuroleptic drugs with hormone-sensitive adenylate cyclase systems, their action on the dopamine-stimulated system appears to be the most potent and to correlate best with the clinical neuroleptic effects of these drugs.

Acknowledgements—The author acknowledges the help given by Dr. L. Iversen during the course of this study. The author is an M.R.C. Scholar.

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