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Trifluoperazine and Chlorpromazine Antagonize *Alpha*$_1$- but Not *Alpha*$_2$-Adrenergic Effects

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SUMMARY

Trifluoperazine and chlorpromazine inhibited in a dose-dependent manner the stimulation of glycogenolysis, gluconeogenesis, and ureogenesis due to *alpha*$_1$-adrenergic stimulation in rat hepatocytes. In contrast, the antipsychotic agents were unable to block the inhibition of adenylate cyclase due to *alpha*$_2$-adrenergic activation in hamster adipocytes. Binding experiments showed that trifluoperazine and chlorpromazine at low concentrations displaced tritiated dihydroergocryptine binding from rat liver membranes (*alpha*$_1$-adrenergic sites), whereas very large concentrations of the phenothiazine derivatives were required to displace dihydroergocryptine from hamster adipocyte membranes (*alpha*$_2$-adrenergic sites). It is concluded that chlorpromazine and trifluoperazine are much more potent at *alpha*$_1$- than at *alpha*$_2$-adrenergic receptors. The use of rat hepatocytes and hamster adipocytes to study the *alpha*-adrenergic subtype selectivity of drugs is proposed.

INTRODUCTION

The antipsychotic phenothiazine drugs, chlorpromazine and trifluoperazine, bind to calmodulin and prevent its activation by calcium. These drugs have been used as tools to study the role of calmodulin in processes in which calcium is known to be involved. However, besides its ability to prevent calmodulin activation, these drugs have local anesthetic, cholinergic, and adrenergic properties. Reinhart *et al.* (1) reported that in perfused rat liver trifluoperazine antagonizes the effects of phenylephrine and suggested that calmodulin was probably involved in the adrenergic effects. Later studies have shown that the effects of trifluoperazine are mainly explained in terms of its effect at the *alpha*-adrenergic receptors (2, 3).

Two basic types of *alpha*-adrenoceptors (*alpha*$_1$ and *alpha*$_2$) have been identified in pharmacological and binding studies (4, 5). We studied the effects of the antipsychotic agents chlorpromazine and trifluoperazine on *alpha*-adrenergic actions and binding in two models: rat hepatocytes and hamster adipocytes. The main adrenergic effects in rat hepatocytes, such as stimulation of glycogenolysis, gluconeogenesis, and ureogenesis, are due to activation of *alpha*$_1$-adrenoceptors (6–8), and about 80% of the adrenoceptors present in their membranes are also of the *alpha*$_1$ subtype (9, 10). On the contrary, about 80% of the *alpha*-adrenoceptors in hamster adipocytes are of the *alpha*$_2$ subtype (11, 12) and mediate inhibitions of adenylate cyclase and lipolysis (11, 13). Our studies show that trifluoperazine and chlorpromazine have a much greater affinity for *alpha*$_1$-adrenoceptors than for *alpha*$_2$-adrenoceptors.

MATERIALS AND METHODS

(−)-Epinephrine, yohimbine, chlorpromazine, urease, and glucose oxidase were obtained from Sigma Chemical Company (St. Louis, Mo.). Bovine serum albumin (Fraction V) and collagenase (Type II) were obtained from Reheis Chemical Company and Worthington Biochemical Corporation (Freehold, N. J.), respectively. [^{3}H]DHE[3] (23 Ci/mole) and [2,8^{3}H]adenosine 3′,5′-cyclic phosphate (32 Ci/nmole) were obtained from New England Nuclear Corporation (Boston, Mass.). The following compounds were generously provided by the sources indicated: trifluoperazine, Smith Kline & French; prazosin, Pfizer; clonidine, Boehringer Ingelheim; and phentolamine, Ciba.

Hepatocytes were isolated from female Wistar rats (180–200 g) by the method of Berry and Friend (14) as modified by Tolbert *et al.* (6). Cells were suspended in Krebs-Ringer bicarbonate buffer (pH 7.4) saturated with O_2/CO_2 (95%:5%) at 37° and supplemented with 1% albumin. Aliquots of the cell suspension [0.5 ml containing 35–45 mg of cells (wet weight)] were added to plastic tubes containing 0.5 ml of the suspending medium and the agents to be tested. Cells were incubated for 60 min in a water bath shaker at 37° under an O_2/CO_2 (95%:5%)

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[3] The abbreviation used is: DHE, dihydroergocryptine.

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atmosphere. The incubation was finished by placing the tubes in an ice bath. Glucose production in the absence of substrates by hepatocytes from fed rats was considered as glycogenolysis. Synthesis of glucose in the presence of 10^{-2} M *l*-lactate by hepatocytes from rats fasted for 24 hr was considered as gluconeogenesis (glucose production in the absence of substrates represented 10% of the total, and it was subtracted). Glucose was determined enzymatically (15). Urea production was quantified in cells from fed rats in medium supplemented with 2 mM ornithine and 10 mM glutamine and was determined by the method of Gutman and Bergmeyer (16).

Golden hamsters (90–100 g) fed ad libitum were used. Adipocytes were obtained by enzymatic digestion of adipose tissue by the method of Hittelman *et al.* (17) as previously described (11). Cyclic AMP accumulation was determined by the method of Gilman (18) at 10 min of incubation in medium containing 10^{-4} M theophylline and adenosine deaminase (0.5 μg/ml).

Rat liver plasma membranes were prepared by the method of Neville (19) as modified by Clarke *et al.* (20). Hamster adipocyte membranes were prepared as described previously (11). Receptor binding assays were performed using [^{3}H]DHE. The assays with liver membranes were performed as described by Clarke *et al.* (20) except that a smaller binding assay of 0.5 ml was used; those with adipocyte membranes were performed as described previously (11), using the procedure of Pecquery *et al.* (21). Nonspecific binding was determined using 10^{-5} M phentolamine. Specific binding constituted 60–80% in the studies with adipocyte membranes and 50–60% in the studies with liver membranes. Membrane protein was determined by the method of Lowry *et al.* (22). EC_{50} is the concentration of antagonist causing half-maximal inhibition of [^{3}H]DHE binding.

RESULTS

In isolated rat hepatocytes epinephrine produced dose-dependent stimulations of glycogenolysis (Fig. 1), gluconeogenesis (Fig. 2), and ureogenesis (Fig. 3). These effects of epinephrine were antagonized by prazosin and the antipsychotic agents; the order of potency was prazosin > chlorpromazine > trifluoperazine (Figs. 1–3). These data confirm the ability of the highly selective *alpha*$_1$-adrenergic antagonist prazosin to block the metabolic effects of epinephrine in rat hepatocytes. Furthermore, they indicate that chlorpromazine and trifluoperazine are potent *alpha*$_1$-antagonists.

Experiments with hamster adipocytes were performed in order to determine whether trifluoperazine and chlorpromazine were able to antagonize *alpha*$_2$-adrenergic effects. Epinephrine (10^{-5} M), an *alpha*- and *beta*-adrenergic agonist, produced a small increase in cyclic AMP levels (from a basal of 49 ± 7 pmoles/10^6 cells to 110 ± 19 pmoles/10^6 cells; data are the means ± standard error of

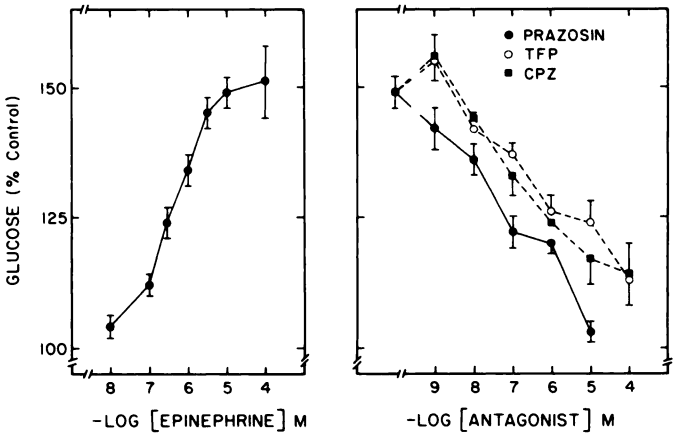

FIG. 2. *Effect of epinephrine on gluconeogenesis in isolated rat hepatocytes and antagonism of its effect by prazosin, trifluoperazine, and chlorpromazine*

Left, hepatocytes were incubated with different concentrations of epinephrine; right, hepatocytes were incubated with 10^{-5} M epinephrine and different concentrations of prazosin, trifluoperazine (*TFP*), or chlorpromazine (*CPZ*). Basal gluconeogenesis was 10 ± 1 nmoles/mg of cells (wet weight). Results are means, and vertical lines represent the standard error of the mean of six or seven different cell preparations.

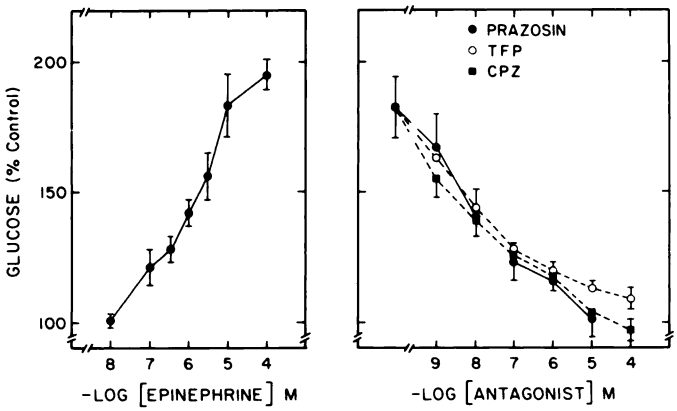

FIG. 1. *Effect of epinephrine on glycogenolysis in isolated rat hepatocytes and antagonism of its effect by prazosin, trifluoperazine, and chlorpromazine*

Left, hepatocytes were incubated with different concentrations of epinephrine; right, hepatocytes were incubated with 10^{-5} M epinephrine and different concentrations of prazosin, trifluoperazine (*TFP*), or chlorpromazine (*CPZ*). Basal glycogenolysis was 53 ± 8 nmoles/mg of cells (wet weight). Results are means, and vertical lines represent the standard error of the mean of six or seven different cell preparations.

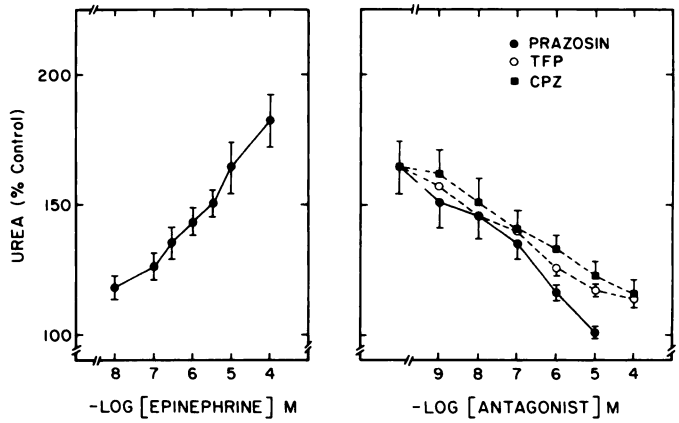

FIG. 3. *Effect of epinephrine on ureogenesis in isolated rat hepatocytes and antagonism of its effect by prazosin, trifluoperazine, and chlorpromazine*

Left, hepatocytes were incubated with different concentrations of epinephrine; right, hepatocytes were incubated with 10^{-5} M epinephrine and different concentrations of prazosin, trifluoperazine (*TFP*), or chlorpromazine (*CPZ*). Basal ureogenesis was 14 ± 2 nmoles/mg of cells (wet weight). Results are means, and vertical lines represent the standard error of the mean of six or seven different cell preparations.

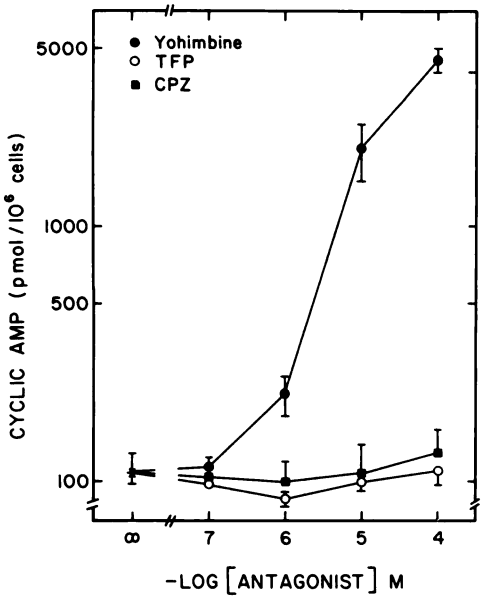

FIG. 4. *Effects of yohimbine, trifluoperazine, and chlorpromazine on the accumulation of cyclic AMP due to epinephrine in hamster adipocytes*

Cells were incubated with 10^{-5} M epinephrine and different concentrations of yohimbine, trifluoperazine (*TFP*), or chlorpromazine (*CPZ*). Results are mean, and vertical lines represent the standard error of the mean of four to eight different cell preparations (note log scales).

the mean of nine experiments in duplicate). Addition of the *alpha*$_2$-adrenergic antagonist yohimbine permitted full expression of the *beta*-adrenergic activity of epinephrine (Fig. 4). Trifluoperazine and chlorpromazine (up to 10^{-4} M) were unable to mimic the effect of yohimbine (Fig. 4). The possibility that the phenothiazine derivatives could have *alpha*$_2$-adrenergic activity was considered. Isoproterenol produced a marked accumulation of cyclic AMP in hamster adipocytes which could be diminished by the addition of the *alpha*$_2$-adrenergic agonist clonidine (Fig. 5). Neither chlorpromazine nor trifluoperazine was able to diminish the accumulation of cyclic AMP due to *beta*-adrenergic activation (Fig. 5). These data suggest that *alpha*$_2$-adrenoceptors have very low affinity for the antipsychotic drugs.

Binding experiments were performed. Saturation experiments with [^{3}H]DHE using liver membranes yielded the following results: B_{max} 525 ± 105 fmoles/mg of protein, K_D 9.35 ± 2.3 nM, and Hill coefficient 0.96 ± 0.01, in agreement with data from other authors (20, 23) (results are the means ± standard error of the mean of four experiments performed in duplicate). Displacement of [^{3}H]DHE binding by prazosin, chlorpromazine, and trifluoperazine in liver membranes is presented in Fig. 6. The potency order was prazosin (EC_{50} 4×10^{-9} M) > chlorpromazine (EC_{50} 1.2×10^{-8} M) > trifluoperazine (EC_{50} 6×10^{-8} M). Competition curves were biphasic, consistent with the presence of two types of adrenoceptors in liver membranes (9, 10) and high selectivity of the drugs (5, 24).

Saturation experiments with [^{3}H]DHE using adipocyte membranes yielded the following results: B_{max} 465 ± 40 fmoles/mg of protein, K_D 5.37 ± 0.29 nM, and Hill coefficient 0.90 ± 0.02, in agreement with previous data (11, 25) (results are the means ± standard error of the mean of three experiments in duplicate). Displacement of [^{3}H]DHE binding by yohimbine, chlorpromazine, and trifluoperazine is presented in Fig. 7. Yohimbine effectively displaced bound [^{3}H]DHE (EC_{50} 5×10^{-7} M), whereas chlorpromazine and trifluoperazine displaced the radioactive ligand only at very high concentrations (EC_{50} 10^{-4} M and 7.5×10^{-5} M, respectively).

The EC_{50} *alpha*$_2$/EC_{50} *alpha*$_1$ ratios were 8300 and 1250 for chlorpromazine and trifluoperazine, respectively, indicating that these drugs have very high selectivity for *alpha*$_1$-adrenergic receptors.

DISCUSSION

A close correlation between the pharmacological and binding was observed. Blackmore and co-workers (2) have shown that the ability of trifluoperazine to inhibit the activation of phosphorylase by epinephrine correlates with its ability to inhibit [^{3}H]epinephrine binding. Our results not only are in agreement with the data of Black-

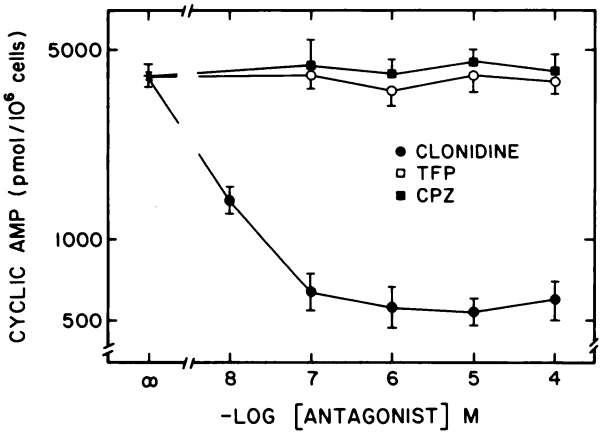

FIG. 5. *Effects of clonidine, trifluoperazine, or chlorpromazine on the accumulation of cyclic AMP due to isoproterenol in hamster adipocytes*

Cells were incubated with 10^{-5} M isoproterenol and different concentrations of clonidine, trifluoperazine (*TFP*), or chlorpromazine (*CPZ*). Results are means, and vertical lines represent the standard error of the mean of four to six different cell preparations (note log scales).

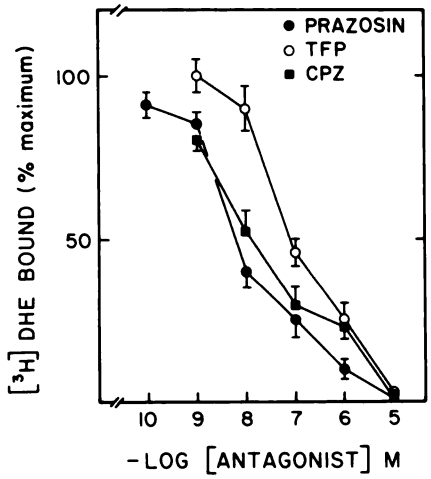

FIG. 6. *Competition curve of prazosin, trifluoperazine (TFP), and chlorpromazine (CPZ) with [^{3}H]DHE in rat liver membranes*

Assays were performed at a [^{3}H]DHE concentration of 1.5 nM. Results are means, and vertical lines represent the standard error of the mean of duplicate determinations with five membrane preparations.

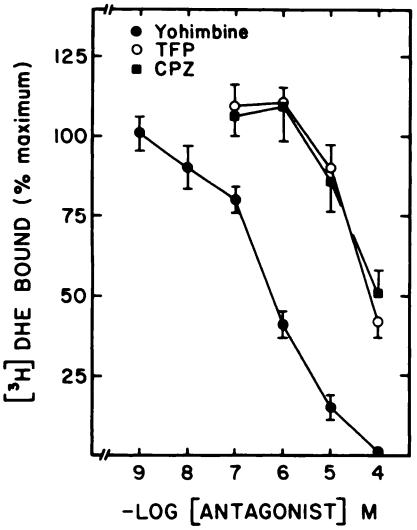

FIG. 7. *Competition curve of yohimbine, trifluoperazine (TFP), and chlorpromazine (CPZ) with [^{3}H]DHE in hamster adipocyte membranes*

Assays were performed at a [^{3}H]DHE concentration of 5 nM. Results are means, and vertical lines represent the standard error of the mean of duplicate determinations with five membrane preparations.

more *et al.* (2) but extend the observation to other *alpha*-adrenergic effects, such as stimulations of gluconeogenesis and ureogenesis, and confirm the involvement of *alpha*$_1$-adrenoceptors in the stimulation of these processes by epinephrine (6–8). Cocks *et al.* (3) have reported that trifluoperazine inhibits the release of K^+ induced by phenylephrine in guinea pig hepatocytes and the contractile response to phenylephrine in rat vas deferens. These authors did not characterize the type of *alpha*-adrenoceptors involved in the responses. Our data suggest that the adrenoceptors involved are of the *alpha*$_1$ subtype. The ability of trifluoperazine and chlorpromazine to inhibit *alpha*$_1$-adrenergic binding obviously precludes their use in investigating the role of calmodulin in *alpha*$_1$-adrenergic actions.

Other authors have studied the selectivity of the phenothiazine agents to displace ligands from *alpha*$_1$- or *alpha*$_2$-adrenoceptors. Thus, U'Prichard *et al.* (26) have reported that chlorpromazine and trifluoperazine are 100-fold more potent in displacing [^{3}H]WB-4101 (an *alpha*$_1$-adrenergic radioligand) than [^{3}H]clonidine (an *alpha*$_2$-adrenergic radioligand) in the central nervous system. Lavin *et al.* (27), on the other hand, reported that chlorpromazine is 400-fold more potent at *alpha*$_1$- than at *alpha*$_2$-adrenoceptors in rabbit uterine membranes as determined by [^{3}H]DHE, [^{3}H]yohimbine, and [^{3}H]prazosin binding. There is agreement between our results and those of U'Prichard *et al.* (26) and Lavin *et al.* (27) in that the antipsychotic agents are more selective for *alpha*$_1$- than for *alpha*$_2$-adrenoceptors, but our data show a much higher selectivity. The reason for this discrepancy is at present unknown, but it might be related to differences in the receptors present in different tissues and species or to technical aspects of membrane preparation and binding assays. In any event, our data suggest that rat hepatocytes and hamster adipocytes may be useful models to determine the selectivity of different drugs for *alpha*-adrenergic subtypes.

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