Biochemical Pharmacology, Vol. 51, pp. 331–337, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
SSDI 0006-2952(95)02187-6

Chlorpromazine Stimulatory Effect on Iron Uptake by Rat Brain Synaptosomes

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ABSTRACT. Clinical long-term neuroleptic administration induces extrapyramidal motor side-effects, of which tardive dyskinesia is the most important. Experimentally, dopamine D_2 supersensitivity is observed after phenothiazine and butyrophenone treatment. Neuroleptic-induced tardive dyskinesia and D_2 modulation have been linked to impaired iron homeostasis in the central nervous system. Increased nonheme iron levels found in the basal ganglia of patients with extrapyramidal symptomology support the connection between iron and neuronal dopaminergic modulation. We now report the effect of chlorpromazine on iron uptake by synaptosomes of rat brain from two different iron donors: [^{55}Fe]citrate and [^{55}Fe]transferrin. Iron uptake from both donors by cortical synaptosomes was stimulated by Ca^{2+} and enhanced by chlorpromazine in a saturable fashion. Synaptosomes from the striatum also showed increased (60%) iron uptake from [^{55}Fe]citrate in the presence of chlorpromazine. Chlorpromazine stimulated iron uptake by cortical synaptosomes more efficiently than Ca^{2+}, at physiological levels, from both [^{55}Fe]transferrin (50%) and [^{55}Fe]citrate (68%). Calcium potentiated the effect of chlorpromazine upon cortical synaptosomal iron uptake from [^{55}Fe]citrate, but had no apparent effect on the uptake from [^{55}Fe]transferrin. Chlorpromazine-stimulated iron uptake from the latter was observed without addition of Ca^{2+}. Moreover, fluorescence measurement of Ca^{2+} uptake by cortical synaptosomes showed intensified uptake in the presence of 50 μM chlorpromazine (42%). Visible spectral studies of chlorpromazine in the presence of Fe^{3+}-citrate and diferric-transferrin did not reveal iron displacement by chlorpromazine from either of the two donors. These data suggest that chlorpromazine may increase iron uptake by neurons, and may be involved in the development of tardive dyskinesia and other extrapyramidal disorders. BIOCHEM PHARMACOL 51;3:331–337, 1996.

KEY WORDS. synaptosomal iron uptake; chlorpromazine; transferrin

Dopaminergic neurotransmission has been suggested to be linked to iron metabolism [1–3]. Furthermore, several reports point out the involvement of iron in the modulation of dopamine D_2 receptors [4, 5]. Tardive dyskinesia, considered to be the most severe side-effect of chronic neuroleptic administration, has been related to the dopamine D_2 receptor supersensitivity promoted by these drugs. Campbell *et al.* [6] reported iron deposition in some cerebral areas of patients with haloperidol-induced tardive dyskinesia, confirming earlier results of Hunter *et al.* [7] and Weiner *et al.* [8] about increased cerebral iron levels after experimental prolonged neuroleptic administration.

According to Ben-Shachar *et al.* [5], rats chronically treated with both CPZ† and $FeCl_2$ do not develop D_2 receptor supersensitivity, as observed when animals are treated chronically with CPZ alone. The authors considered the ability of iron to abolish the CPZ-induced increased D_2 receptor density as being due to the reduced neuroleptic concentration in the brain promoted by iron, which is attributed to the fact that neuroleptics are potent iron-chelating agents [9]. Moreover, Ben-Shachar and Youdim [4] demonstrated that iron-deficient animals develop an extensive dopaminergic supersensitivity with increased D_2 receptor density upon haloperidol and CPZ treatment. Recently Ben-Shachar *et al.* [10] reported $^{59}FeCl_3$ transport facilitation by phenothiazines into brain, not verified with clozapine, an atypical neuroleptic not associated with significant extrapyramidal side-effects or D_2 supersensitivity. Altogether, these findings have been interpreted as resulting from alterations in the blood-brain barrier transport of neuroleptics related to iron availability.

Iron transport across the blood-brain barrier via transferrin receptors located on the brain microvasculature, as well as its distribution in the brain [11], have been characterized. Swaiman and Machen [12] showed iron uptake by cortex neurons, and Giometto *et al.* [13], using immunocytochemical techniques, demonstrated the presence of transferrin receptors on neurons.

In this study, we examined the capacity of iron uptake from [^{55}Fe]transferrin and [^{55}Fe]citrate by the synaptic neuronal portion (synaptosomes) in the presence and absence of CPZ. For comparative purposes, synaptosomal preparations from striatum and cortex tissue were used. The former is rich in dopaminergic nerve terminals and the latter is unrelated

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† *Abbreviations:* CPZ, chlorpromazine; NTA, nitrilotriacetic acid; PPO, 2,5-diphenyloxazole; and POPOP, 1,4-bis-[4-methyl-5-phenyl-2-oxazolyl] benzene.

Received 24 February 1995; accepted 14 August 1995.

to dopaminergic innervation and rich in transferrin receptors [11, 13].

MATERIALS AND METHODS

Materials

$^{55}FeCl_3$ (4.51 mCi/mmol) was purchased from Amersham. Apotransferrin, CPZ, Fura 2-AM, NTA, PPO and POPOP were obtained from Sigma and GF/B filters from Whatman. All other reagents were of analytical grade. Stock solutions were prepared with Millipore MilliQ deionized water.

Synaptosomal Preparation

Synaptosomes were prepared according to Cotman and Matthews [14] from cerebral cortex or striatum, as specified in Results. Two-month-old rats (180–220 g) were killed by cervical dislocation; each brain was removed and the tissue immediately dissected at about 4° for synaptosomal preparation. Protein concentration was determined by the method of Lowry *et al.* [15]. The time window for using synaptosomes viable for iron uptake, stored at 4°, was found to be 2 days.

[^{55}Fe]Citrate and [^{55}Fe]Transferrin Assay

Synaptosomes were diluted to a final concentration of 0.5 mg/mL in pure O_2-purged 10 mM HEPES buffer, pH 7.4, containing: 124 mM NaCl, 5 mM KCl, 1.24 mM KH_2PO_4, 1.3 mM $MgCl_2$, 1.2 mM $CaCl_2$ and 10 mM glucose. Synaptosomal samples were incubated in this medium at 37° for 15 min prior to the addition of [^{55}Fe]citrate or [^{55}Fe]transferrin. The concentrations, addition, and incubation times of the [^{55}Fe]species are specified in the legends to the figures. The incubation was stopped by transferring 450-μL aliquots of incubation mixture to 3 mL of ice-cold buffer, and the samples were filtered rapidly through Whatman GF/B filters in a Millipore multifold system apparatus. The filters were washed twice with 3 mL of buffer, dried, transferred to the scintillation fluid (0.5% PPO plus 0.01% POPOP dissolved in toluene), and counted in a Tricarb Packard Liquid Scintillation Analyzer. The molar concentration of radiolabeled iron was estimated by direct counting of [^{55}Fe]species-containing aliquots. Nonspecific counting ([^{55}Fe]species non-synaptosome-associated) was determined for each different addition by incubating [^{55}Fe]species-containing aliquots in the absence of tissue. This blank contributes only 10% of the total counts. Iron uptake was determined as the difference between counts in the presence and absence of synaptosomes. Results are expressed as iron equivalents per milligram of protein per incubation time.

Since this methodological approach refers to total synaptosome-associated [^{55}Fe]species (uptake plus membrane binding), sonicated synaptosomal samples were prepared in order to evaluate the extent of actual [^{55}Fe]citrate uptake, distinguishable from membrane [^{55}Fe]citrate binding. Synaptosomes were incubated in the presence of increasing concentrations of [^{55}Fe]citrate for 15 min at 37°. After incubation, the samples were washed two times by centrifugation at maximum speed in an Eppendorf microcentrifuge in order to eliminate soluble external iron. After resuspension of the final pellet, samples were sonicated and centrifuged, and aliquots of the supernatant were taken for counting. The iron uptake concentration was determined by the difference between supernatant counting of sonicated samples and the counting of non-sonicated synaptosomal samples. The latter represents the total iron associated with synaptosomes (membrane bound and uptake). By this method, iron uptake levels were found to represent *ca.* 40% of the total synaptosome-associated iron. According to these control assays, we may safely conclude that there is synaptosomal iron internalization from [^{55}Fe]citrate as donor. Data shown in all the figures refer to iron uptake concentrations obtained by the filtration method. [^{55}Fe]transferrin uptake was not evaluated by sonicating samples. Since iron uptake from [^{55}Fe]transferrin is dependent on high-affinity membrane–receptor interaction, the [^{55}Fe]transferrin synaptosome association shown here probably is due to a transferrin-receptor-mediated process.

[^{55}Fe]Citrate was obtained by mixing $^{55}FeCl_3$ aliquots with sodium citrate immediately before assay (molar ratio of $^{55}FeCl_3$:citrate was 1:4). [^{55}Fe]Loaded transferrin was obtained according to Konopka and Romslo [16]. Apotransferrin was dissolved in 10 mM HEPES buffer, pH 7.4, containing 10 mM $NaHCO_3$ and 0.1 mM $NaClO_4$. $^{55}FeCl_3$ was dissolved in NTA (120% $^{55}FeCl_3$ to NTA), and the complex [^{55}Fe]NTA was transferred to the apotransferrin-containing solution. The final solution was dialyzed against 20 mM $NaHCO_3$ overnight. The diferric-transferrin absorbance was read at 466 and 280 nm and identified by the ratio A_{466}/A_{280} (0.045, according to Nilsen [17]).

Calcium Uptake Determination

These measurements were performed with cortical synaptosomes (0.5 mg protein/mL) incubated in the same buffer used for the [^{55}Fe]citrate and [^{55}Fe]transferrin assays. Calcium uptake was determined by fluorescence measurements with the probe Fura 2-AM as described by Nachshein [18]. Synaptosomal preparations previously loaded with 3 μM Fura 2-AM were incubated for 15 min at 37° in medium containing 1.0 mM $CaCl_2$, in the presence or absence of 50 μM CPZ. The excitation spectra were recorded (300–400 nm) on a SPEX (Fluorolog®) spectrofluorimeter.

RESULTS

Iron uptake by synaptosomes from both [^{55}Fe]citrate and [^{55}Fe]transferrin increased with time, reaching saturation at *ca.* 15 and 20 min, respectively (Fig. 1). Synaptosomal iron uptake from [^{55}Fe]citrate and [^{55}Fe]transferrin at increasing concentrations of external Ca^{2+} was measured after a 15-min incubation (Fig. 2). Similar to the Ca^{2+}-stimulated iron uptake by hepatocytes [17], the stimulating effect of Ca^{2+} on synaptosomal iron uptake from chlorpromazine reached a plateau at *ca.*

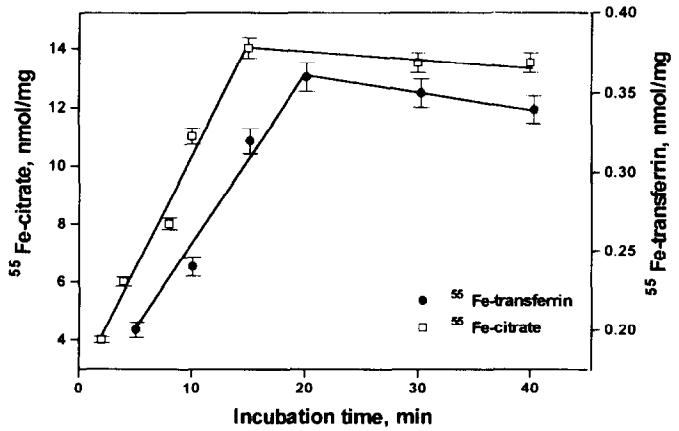

FIG. 1. Time course of cortex synaptosomal iron uptake from [^{55}Fe]citrate and [^{55}Fe]transferrin. Synaptosomes (0.5 mg/mL) resuspended in 10 mM HEPES buffer, pH 7.4, containing 124 mM NaCl, 5 mM KCl, 1.24 mM KH_2PO_4, 1.3 mM $MgCl_2$, 1.2 mM $CaCl_2$ and 10 mM glucose, were incubated at 37° in the presence of 15 μM [^{55}Fe]citrate or 15 μM [^{55}Fe]transferrin. At the times indicated, triplicate aliquots were withdrawn, and iron uptake was determined. Results are means ± SD (N = 9) of three independent experiments for identical [^{55}Fe]species concentrations.

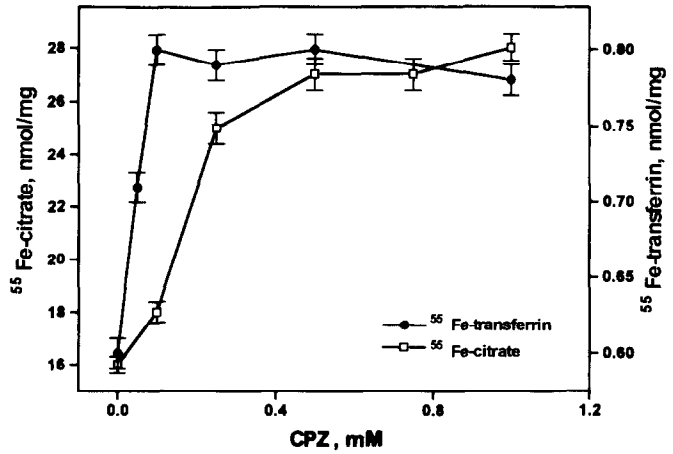

FIG. 3. Effect of increasing concentrations of CPZ on cortex synaptosomal iron uptake from [^{55}Fe]citrate and [^{55}Fe]transferrin. Synaptosomes were incubated as described in the legend of Fig. 1, in medium containing 15 μM [^{55}Fe]citrate or 30 μM [^{55}Fe]transferrin and CPZ at the indicated concentrations. After a 15-min incubation, triplicate aliquots were withdrawn, and iron uptake was determined. Results are means ± SD (N = 9) of three independent assays.

1.0 mM Ca^{2+}. Iron uptake from [^{55}Fe]transferrin reached saturation at lower Ca^{2+} concentrations (about 0.5 mM).

The effect of CPZ on iron uptake from [^{55}Fe]citrate and [^{55}Fe]transferrin was investigated. Cortical synaptosomes incubated at increasing CPZ concentrations showed increased iron uptake from both iron species (Fig. 3). Iron uptake from [^{55}Fe]transferrin reached saturation at 0.1 mM CPZ versus 0.5 mM from [^{55}Fe]citrate. Iron uptake from [^{55}Fe]citrate in the presence of 0.25 mM CPZ increased rapidly and linearly for 10 min, reaching the highest control value (synaptosomes incubated in the absence of CPZ) within less than 8 min (Fig. 4). Iron uptake from [^{55}Fe]citrate by cortex synaptosomes, incubated in the presence of 0.25 mM CPZ and assayed by sonicating synaptosomal samples (see Materials and Methods), was found to be 45% higher than that obtained with sonicated control samples (without CPZ; Table 1). This value was slightly lower than that obtained by the filtration method, where 0.25 mM CPZ intensified iron uptake from [^{55}Fe]citrate

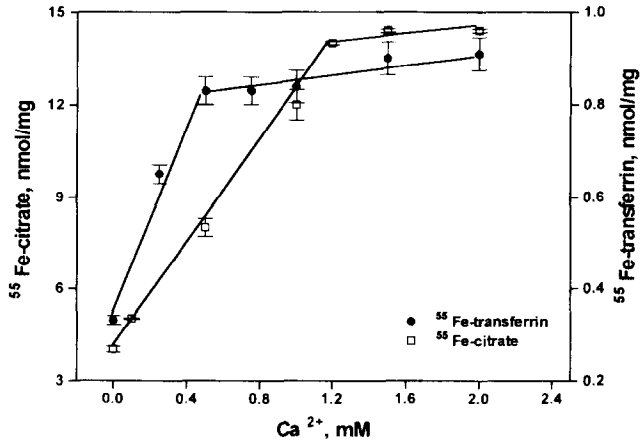

FIG. 2. Effect of increasing concentrations of Ca^{2+} on cortex synaptosomal iron uptake from [^{55}Fe]citrate and [^{5}Fe]transferrin. Synaptosomes were incubated as described in the legend of Fig. 1, in medium containing 15 μM [^{55}Fe]citrate or 30 μM [^{55}Fe]transferrin and Ca^{2+} at the indicated concentrations or without the addition of Ca^{2+} (contaminant Ca^{2+} is supposed to be at the 0–10 μM range). After 15-min incubation, triplicate aliquots were withdrawn, and iron uptake was determined. Results are means ± SD (N = 9) of three independent assays.

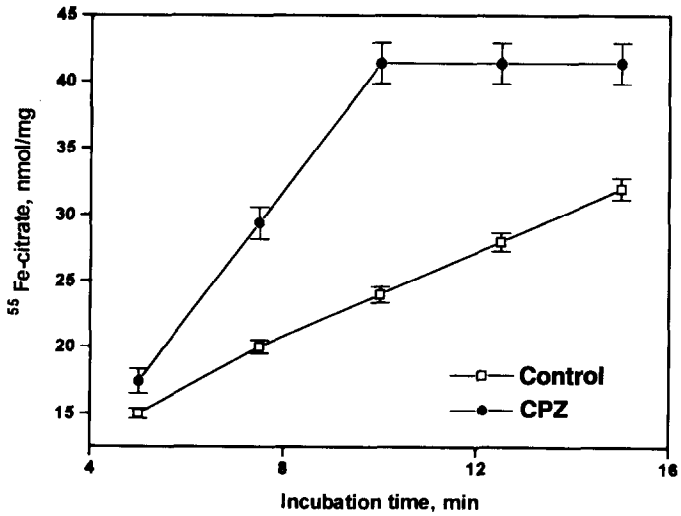

FIG. 4. Time course of CPZ effect on cortex synaptosomal iron uptake from [^{55}Fe]citrate. Synaptosomes were incubated as described in the legend of Fig. 1, in medium containing 30 μM [^{55}Fe]citrate, in the absence (control) or presence of 0.25 mM CPZ. At the times indicated, triplicate aliquots were withdrawn, and iron uptake was determined. Results are means ± SD (N = 6) and represent two independent experiments.

TABLE 1. Cortex synaptosomal iron uptake from [^{55}F]citrate obtained by sonicating synaptosomal samples

Synaptosomal concentration (mg/mL)	Iron uptake (nmol/mg/15 min)	
	Control	CPZ (50 μM)
1	6.4 ± 0.5	9.5 ± 0.6*
2	13.0 ± 0.7	17.0 ± 0.5*

Synaptosomes were incubated as described in the legend of Fig. 1, in medium containing 15 μM [^{55}Fe]citrate. After a 15-min incubation, samples were centrifuged two times, the final pellets were resuspended, sonicated, and centrifuged, and aliquots of the supernatant were taken for counting as described in Materials and Methods. Results are means ± SD (N = 6) of two independent assays performed in triplicate.

* $P < 0.001$.

by 62% (Fig. 3). However, CPZ stimulation of actual iron uptake from [^{55}Fe]citrate and not just membrane binding was confirmed by this experiment. Sonicated control samples were shown to take up only 40% of the iron (6.4 nmol/mg/15 min; Table 1) determined by the filtration method (15 nmol/mg/15 min; Fig. 1), at the same [^{55}Fe]citrate concentration (15 μM) in the medium. Table 1 shows that synaptosomal iron uptake responds to the protein concentration. Iron uptake from [^{55}Fe]transferrin by sonicated synaptosomal samples were not determined.

The effect of external Ca^{2+} concentration in the presence of CPZ on iron uptake by synaptosomes was also studied. As depicted in Fig. 5, iron uptake from [^{55}Fe]citrate in the presence of 0.25 mM CPZ, at increasing Ca^{2+} concentrations, was higher than that observed in CPZ-free medium (control samples). Synaptosomal iron uptake from [^{55}Fe]citrate in Ca^{2+}-free medium containing 0.25 mM CPZ was as high as that observed

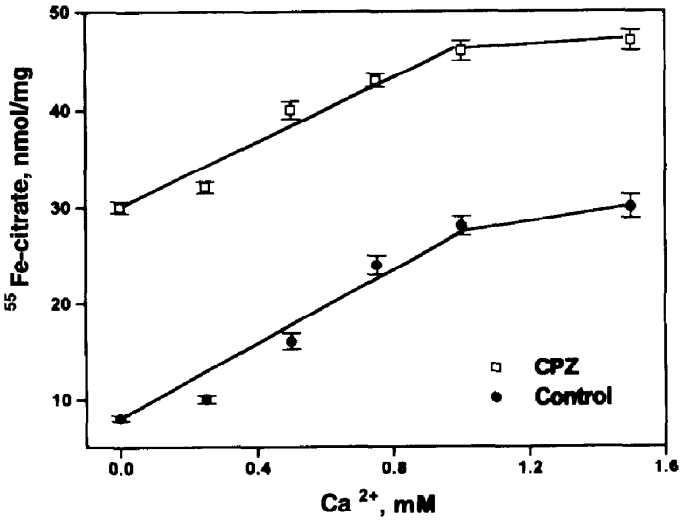

FIG. 5. Effect of increasing Ca^{2+} concentrations on cortex synaptosomal iron uptake from [^{55}Fe]citrate in the presence of CPZ. Synaptosomes were incubated as described in the legend of Fig. 1, in medium containing 30 μM [^{55}Fe]citrate, in the absence (control) or presence of 0.25 mM CPZ. Calcium was added at the indicated concentrations. After a 15-min incubation, triplicate aliquots were withdrawn, and iron uptake was determined. Results are means ± SD (N = 6) of two independent experiments.

in the presence of 1.0 mM Ca^{2+} without CPZ (Fig. 5). These data indicate that Ca^{2+} potentiates the CPZ effect on iron uptake from [^{55}Fe]citrate. Similar experiments with [^{55}Fe]-transferrin did not reveal significant alterations in iron uptake at increasing Ca^{2+} concentrations (Table 2), but iron uptake in the presence of 0.1 mM CPZ and the absence of added Ca^{2+} was slightly higher (0.59 nmol/mg/15 min) than that observed for control samples (CPZ-free) in the presence of 1.0 mM Ca^{2+} (0.44 nmol/mg/15 min). The effect of chlorpromazine upon Ca^{2+} uptake was also studied in an attempt to elucidate CPZ-Ca^{2+} potentiation of iron uptake. As depicted in Fig. 6, 50 μM CPZ increased synaptosomal Ca^{2+} uptake by 42%.

Experiments with synaptosomes from striatum also showed CPZ-stimulated iron uptake from [^{55}Fe]citrate, similar to that presented by synaptosomes of cortex origin. Iron uptake from [^{55}Fe]citrate increased *ca.* 60% in the presence of 0.25 mM CPZ (Table 3). Chlorpromazine (0.1 mM) had no effect on striatum synaptosomal iron uptake from [^{55}Fe]transferrin (Table 3). When comparing the iron uptake ability of striatum and cortex synaptosomes, we found that the former takes up iron *ca.* 10-fold less efficiently than the latter (Fig. 7). Accordingly, the transferrin receptor density is known to be highly distributed in cortex neurons and only moderately in striatum structures [11, 13].

Possible CPZ-Fe^{3+} interactions were evaluated by spectral absorption studies. The diferric-transferrin peak at 466 nm was not altered upon addition of CPZ in the range of 50 μM to 1 mM (Fig. 8); iron-loaded transferrin was obtained with $FeCl_3$ as described for [^{55}Fe]transferrin. Furthermore, the diferric-transferrin absorbance ratio at 466 and 280 nm was not altered in the presence of 1 mM CPZ at slightly acidic pH, during 24 hr of incubation ($A_{466}/A_{280} = 0.045$). Nilsen [17] reported this same value for the A_{466}/A_{280} ratio of diferric-transferrin alone. These results show that CPZ, at that concentration range, is not capable of displacing iron from transferrin. Spectral studies of CPZ in the presence of $FeCl_3$ as well as Fe^{3+}–citrate complex were also performed with the aim of studying any CPZ–iron association (Fig. 9). Absorption of the CPZ–iron complex at 524 nm was then verified in the presence of $FeCl_3$, as previously described by Borg and Cotzias [9]. On the other hand, when CPZ was incubated at increasing concentrations (150 μM to 1.5 mM) of Fe^{3+}–citrate complex for 2 hr at 37°,

TABLE 2. Cortex synaptosomal iron uptake from [^{55}Fe]transferrin at increasing Ca^{2+} concentrations

Samples	[Ca^{2+}] (mM)	Iron uptake (nmol/mg/15 min)
Control	1.0	0.44 ± 0.40
Plus 0.1 mM CPZ	0	0.59 ± 0.50*
	0.25	0.55 ± 0.50
	0.5	0.56 ± 0.45
	1.0	0.58 ± 0.35

Synaptosomes were incubated as described in the legend of Fig. 1, in medium containing 15 μM [^{55}Fe]transferrin. After a 15-min incubation, triplicate aliquots were withdrawn, and iron uptake was determined. Results are means + SD (N = 6) of two independent assays.

* $P < 0.01$.

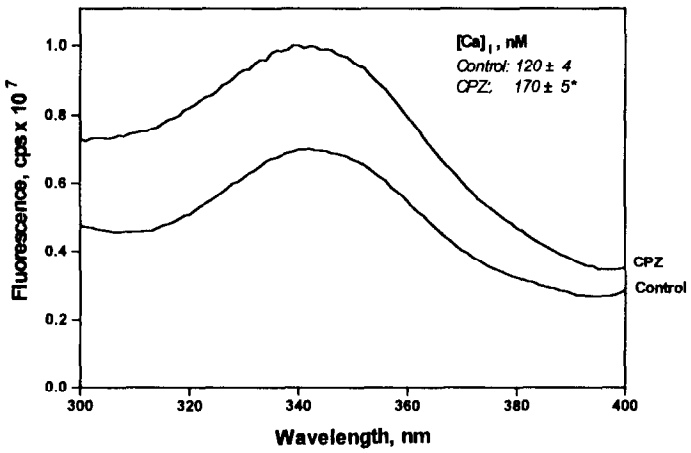

FIG. 6. Representative Fura 2/Ca^{2+} complex spectra in a cortical synaptosomal preparation incubated in the presence of CPZ. The synaptosomal preparation (0.5 mg/mL) was resuspended in 10 mM HEPES buffer, pH 7.4, containing: 124 mM NaCl, 5 mM KCl, 1.24 mM KH_2PO_4, 1.3 mM $MgCl_2$ and 10 mM glucose. After loading Fura 2-AM, samples were incubated in the presence of 1 mM $CaCl_2$ (control samples) and 1 mM $CaCl_2$ plus 50 μM CPZ (CPZ samples) for 15 min at 37°. Fluorescence was measured at 470 nm. Intrasynaptosomal Ca^{2+} concentration ($[Ca]_i$) was calculated as described by Nachshein [18]; results are the means ± SD (N = 9) of three independent experiments. Key: (*) $P < 0.001$.

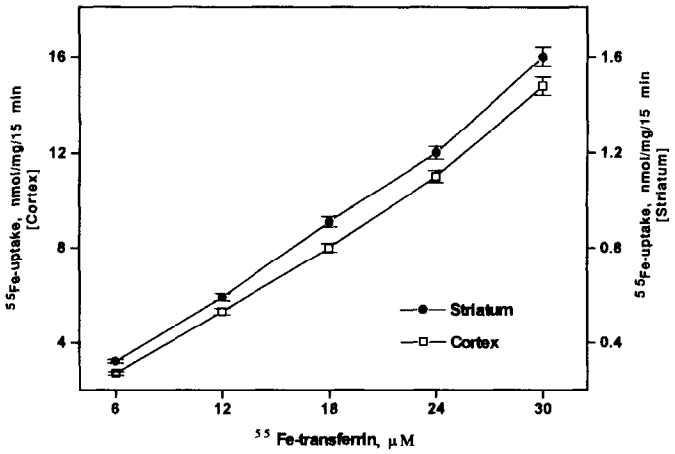

FIG. 7. Synaptosomal iron uptake at increasing [^{55}Fe]transferrin concentrations. Synaptosomes were incubated as described in the legend of Fig. 1, at the indicated [^{55}Fe]transferrin concentrations. After a 15-min incubation, triplicate aliquots were withdrawn, and iron uptake was determined. Results are means ± SD (N = 6) of two independent assays.

no absorption band at 524 nm appeared, indicating no formation of CPZ–iron complex. These spectral studies indicate that citrate- as well as transferrin-bound iron cannot be displaced by CPZ, despite the CPZ "free" iron chelating ability.

DISCUSSION

The present study demonstrates that there is iron uptake by synaptosomes, Ca^{2+}-stimulated and intensified by CPZ in a saturable fashion.

According to Nilsen [17], the extracellular Ca^{2+}-stimulated iron uptake by hepatocytes from different iron donors (ferrous and ferric non-protein species and transferrin) indicates the involvement of the same mechanism for all donors. As attested to in the present study, external Ca^{2+} also stimulates synaptosomal iron uptake from [^{55}Fe]citrate and [^{55}Fe]transferrin. Maximal Ca^{2+}-stimulated iron uptake by synaptosomes occurred at Ca^{2+} levels below physiological concentrations with transferrin as iron donor and at concentrations near the physiological levels with iron–citrate (Fig. 2). Addition of 1.2 mM Ca^{2+} to synaptosomes incubated in a Ca^{2+}-free medium for 15 min immediately and almost completely restored the ability of the synaptosomes to take up iron from [^{55}Fe]transferrin: iron uptake in Ca^{2+}-free medium was 0.3 nmol/mg/15 min and after addition of 1.2 mM $CaCl_2$, iron uptake increased to 0.75 nmol/mg/15 min. This indicates that synaptosomal [^{55}Fe]transferrin uptake, contrary to reticulocytes [19] but similar to hepatocytes [17], is not impaired irreversibly by Ca^{2+} depletion. Using [^{125}I]transferrin, Nilsen [17] concluded that Ca^{2+} does not stimulate transferrin binding to the membrane.

TABLE 3. Striatum synaptosomal iron uptake in the presence of CPZ

	Iron uptake (nmol/mg/15 min)	
[^{55}Fe]species	Control samples	CPZ samples
[^{55}Fe]Citrate (15 μM)	9.7 ± 0.7	16.0 ± 0.8*
[^{55}Fe]Transferrin (30 μM)	1.50 ± 0.50	1.60 ± 0.55

Synaptosomes were incubated as described in the legend of Fig. 1, in the presence of 0.25 mM CPZ (in the case of [^{55}Fe]citrate) and 0.1 mM CPZ (in the case of [^{55}Fe]transferrin). After a 15-min incubation, triplicate aliquots were withdrawn, and iron uptake was determined. Results are means ± SD (N = 6) of two independent assays.

* $P < 0.001$.

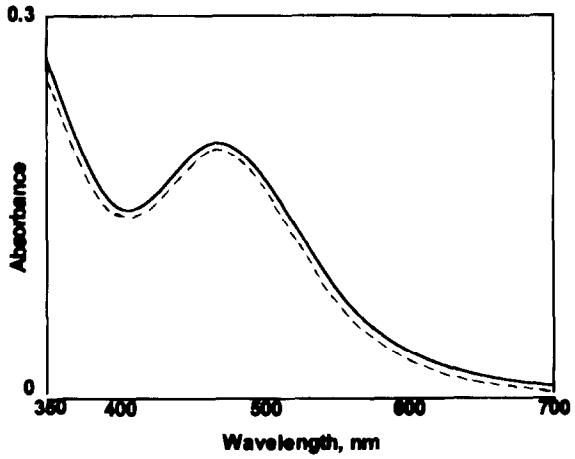

FIG. 8. Visible absorption spectrum of diferric-transferrin in the presence of CPZ. Absorption spectra of 30 μM diferric-transferrin in 10 mM HEPES buffer, pH 7.4, at 37°, in the absence (- - -) and the presence (——) of either 50 μM, 400 μM or 1 mM CPZ, recorded immediately after CPZ addition or 24 hr afterwards. Spectra were traced in a Hitachi double-beam spectrophotometer.

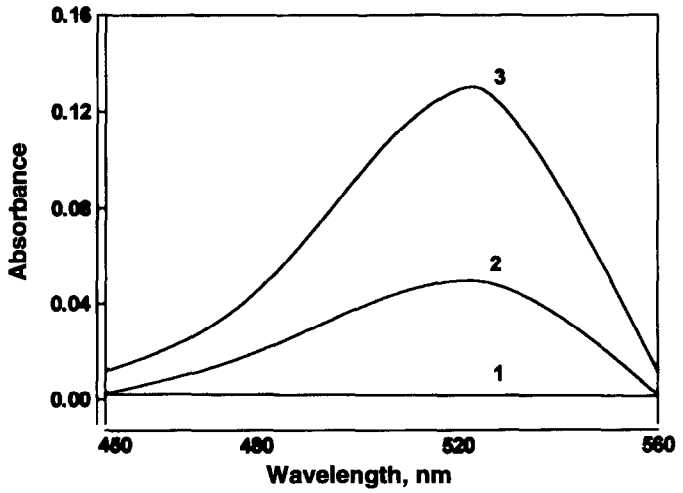

FIG. 9. Visible absorption spectrum of CPZ in the presence of iron species. Chlorpromazine (50 μM) spectra were traced upon addition of: (1) 1.5 mM Fe^{3+}–citrate, either immediately or 2 hr after Fe^{3+}–citrate addition; (2) 0.5 mM $FeCl_3$; and (3) 1.5 mM $FeCl_3$. Spectra 2 and 3 were recorded immediately after $FeCl_3$ addition. CPZ alone does not absorb at this wavelength range. Experimental conditions were described in the legend of Fig. 8.

Thus, calcium was presumed to regulate transmembrane iron transport mechanisms.

Chlorpromazine-stimulated iron uptake by synaptosomes from both iron donors studied here suggests a common mechanism underlying the CPZ effect. Since CPZ did not displace iron from either the Fe^{3+}–citrate complex or diferric–transferrin (Figs. 8 and 9), it seems that CPZ-stimulated iron uptake does not depend on a direct CPZ–iron interaction. One might raise the possibility that CPZ facilitates the binding of iron donors to membrane-specific sites such as the transferrin receptors. To confirm this possibility, however, further studies of the effect of CPZ on transferrin binding are necessary. Chlorpromazine interference on iron transmembrane transport processes might also be considered. Since the effect of CPZ on iron uptake from transferrin in Ca^{2+}-depleted medium was comparable to that observed in CPZ-free medium in the presence of physiological Ca^{2+} levels (Table 2), we suggest that CPZ affects the transmembrane transport of iron complexes, perhaps similarly to the Ca^{2+} mechanism. The findings that CPZ stimulates synaptosomal Ca^{2+} uptake (Fig. 6) and increasing Ca^{2+} concentrations in the presence of CPZ (Fig. 5) linearly augment the iron uptake from [^{55}Fe]citrate suggest no Ca^{2+}-CPZ competition, but rather a synergistic effect. Many authors report strong CPZ/membrane interactions [20, 21] attributed to the highly hydrophobic CPZ structure. Thus, we raise the possibility that the CPZ-stimulated iron uptake studied here may be correlated to CPZ-promoted increase of the membrane permeability. Chlorpromazine-stimulated iron and Ca^{2+}-stimulated uptake (Figs. 3 and 6) might be indicative of CPZ facilitation of transmembrane processes. Further investigation is necessary to elucidate the mechanism by which CPZ stimulates iron and calcium uptake by synaptosomes.

Citrate is believed to be one of the major components of the non-transferrin bound iron pool. It binds iron when transferrin is fully saturated [22]. The Fe^{3+}–citrate complex forms a spherical polymer, at physiological pH, which has been proposed to exhibit structural similarities to the iron core in the ferritin molecule [22]. This polymer can contain about 1.5×10^3 atoms of iron [23, 24]. Thus, it is not surprising that iron uptake from [^{55}Fe]citrate was much more efficient than from [^{55}Fe]transferrin (Fig. 1), even assuming that this uptake is not receptor-mediated.

The present results clearly demonstrate that CPZ-enhanced iron uptake by synaptosomes is not related to dopamine–receptor interaction, since the cortex tissue is poor in dopaminergic nerve terminals. As observed, iron uptake by striatal synaptosomes showed results similar to those observed for cortex synaptosomes when [^{55}Fe]citrate was used as the iron donor (Table 3). However, the iron uptake data with [^{55}Fe]transferrin and striatal synaptosomes in the presence of CPZ significantly differed from those obtained with cortex samples as suggested by the lack of effect of 0.1 mM CPZ on iron uptake (Table 3).

The reported effect of iron on D_2-receptor modulation [5], as well as iron accumulation in brain upon prolonged CPZ treatment [8], might be associated with CPZ–iron uptake interaction, which may account for a lower CPZ availability for dopamine D_2 receptor binding and increased iron uptake by the brain during CPZ administration.

Spectral absorption studies clearly indicated that CPZ, under our experimental conditions, has no ability to displace iron from both iron donors (Figs. 8 and 9). Therefore, the iron uptake in the presence of CPZ reported here seems to be unrelated to direct CPZ–iron interaction as verified in the presence of $FeCl_3$. At physiological conditions, direct CPZ–iron interaction is unlikely to occur, since the iron pools are supposed to be completely protein bound.

Further investigation with CPZ-related neuroleptics and atypical compounds will be valuable to elucidate the importance of CPZ–iron uptake association on neuroleptic-induced tardive dyskinesia. In conclusion, our data suggest that the observed extrapyramidal symptomology and iron increased levels in brain after CPZ prolonged treatment may be associated with CPZ-stimulated iron uptake by neurons.

This work was supported by the Conselho National de Desenvolvimento Científico e Tecnológico, CNPq, the Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP, and the Programa de Apoio ao Desenvolvimento Científico e Tecnológico, PADCT. We thank Dr. Frank Quina (Instituto de Química, USP) for reading this manuscript.

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