The marked difference in the selectivity of these H_1-receptor antagonists for the two monoamine uptake systems suggests that these effects are not secondary to the well known membrane-stabilising properties of the H_1-antihistamines [8]. Indeed it was notable that the H_1-antagonists inhibited amine uptake to the same maximal extent as imipramine and did not affect the imipramine-insensitive uptake over the concentration range employed. However, in general the concentrations of these drugs required to inhibit monoamine uptake systems are much higher than those required for occupancy of histamine H_1-receptors [9]. Nevertheless, the uptake inhibiting properties of these drugs may well contribute to their pharmacological activity *in vivo* where the concentration of these H_1-receptor antagonists may reach the range at which noradrenaline and 5-HT uptake is inhibited. In this respect it is important to note that histamine can induce a release of noradrenaline and 5-HT from certain central and peripheral tissues via a non-receptor tyramine-like action [5, 10–12]. These effects can be prevented by inhibitors of monoamine uptake [10, 11]. Consequently, if the *in vivo* concentrations of H_1-antihistamines reach the micromolar range then many of the H_1-receptor antagonists tested here will inhibit this non-receptor action of exogenous histamine. This has important implications for the interpretation of *in vivo* studies of the action of histamine in the CNS.

In summary, the present study shows that a range of structurally diverse H_1-receptor antagonists can inhibit the uptake of both 5-HT and noradrenaline in the CNS. Many of these agents show selectivity towards one particular monoamine transport system. The finding that at low concentrations (+)-chlorpheniramine is a potent and relatively selective inhibitor of 5-HT transport suggests that this agent may inhibit the neuronal uptake of 5-HT at the therapeutic doses required to achieve H_1-receptor antagonism. These data therefore raise questions concerning the pharmacological consequences of chronic administration of this popular antihistamine and the therapeutic potential of these effects. For example, (+)-chlorpheniramine may have an additional therapeutic application in the treatment of depression if selective 5-HT uptake blockade underlies the clinical efficacy of antidepressants such as zimelidine [13] and fluoxetine [14].

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Department of Physiology and Pharmacology
Medical School
Queen's Medical Centre
Clifton Boulevard
Nottingham NG7 2UH, U.K.

CATHERINE S. YOUNG
ROBERT MASON
STEPHEN J. HILL*

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* Author for correspondence.

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Cyclopiazonic acid inhibition of the Ca^{2+}-transport ATPase in rat skeletal muscle sarcoplasmic reticulum vesicles*

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Cyclopiazonic acid (Fig. 1), a mycotoxin produced by certain fungi of the *Aspergillus* and *Penicillium* genera, may be found as a natural contaminant of corn, peanuts and certain mold-fermented cheeses and meats [1]. Although the clinical signs of toxicity are species dependent, the skeletal muscle is frequently involved and symptoms include muscular incoordination and altered motor activity [2, 3]. It has been suggested that cyclopiazonic acid toxicity may be due to a direct effect on the muscle [4]. The following studies were conducted to investigate the effect of cyclopiazonic acid on the Ca^{2+}-transport ATPase found

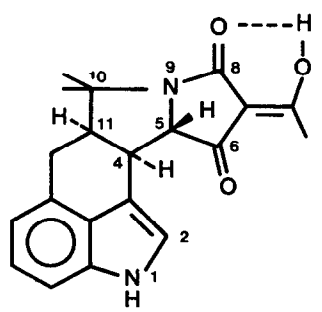

Fig. 1. Structure of cyclopiazonic acid.

* The mention of a trademark, vendor or proprietary name does not imply its preference by the U.S. Department of Agriculture to the exclusion of others that may also be suitable.

in sarcoplasmic reticulum (SR) vesicles isolated from rat skeletal muscle. This transport protein plays an integral role in muscle contraction [5, 6]. The results presented here are the first to show that cyclopiazonic acid is a potent inhibitor of both Ca^{2+} transport and Ca^{2+}-dependent ATPase activity of SR vesicles isolated from rat skeletal muscle.

Materials and methods

Materials. Crystalline Na^+-ATP from equine muscle (4 ppm Ca^{2+} and substantially vanadium free), arsenazo III, 3-[*N*-morpholino]-propanesulfonic acid (MOPS), calcium ionophore A23187, ethylene glycol bis-(β-aminoethyl ether) *N,N,N',N'*-tetra-acetic acid (EGTA), and histidine were purchased from the Sigma Chemical Co. (St. Louis, MO) and $^{45}CaCl_2$ was from the Amersham Corp. (Arlington Heights, IL). Cyclopiazonic acid, 99% pure based on its UV extinction coefficient at 284 nm [7], was produced and purified from cultures of *P. griseofulvum.*

Sarcoplasmic reticulum vesicle preparation. Skeletal muscle SR vesicles were prepared from mature male Sprague–Dawley rats fed Agway certified diet RMH-3000 (Agway Inc., Ithaca, NY) and maintained on a 12-hr light–dark cycle. The rats were anesthetized with ethyl ether prior to decapitation. The crude intermediate membrane fraction was isolated using a procedure modified from those reported by others [8, 9]. Muscle from the hind leg was minced with scissors and homogenized in 3 vol. of 0.1 M KCl, 2 mM EDTA and 5 mM phosphate, pH 7.0, for 5 min with a Polytron, followed by 1 min in a Waring blender. The homogenate was centrifuged at 6,500 *g* for 15 min; the supernatant fraction was filtered through cheese cloth and centrifuged at 10,000 *g* for 15 min; and the SR vesicles were pelleted at 45,000 *g* for 1 hr. The pellet was suspended by hand with a Dounce tissue grinder in a volume of 0.6 M KCl, 10% sucrose and 5 mM histidine, pH 7.0, equal to 40% of the initial tissue weight. After a 30-min incubation, the suspension was centrifuged at 10,000 *g* for 15 min, and the SR vesicles were pelleted at 45,000 *g* for 1 hr, resuspended in 10% sucrose and 5 mM histidine, pH 7.0, equal to 20% of the tissue weight (v/w), and repelleted as above. The final pellet was suspended in the sucrose–histidine buffer in a volume equal to 5% of the tissue weight, divided into aliquots, and frozen at −80° until use. All isolation procedures were performed at 4°. Final protein concentration was between 10 and 15 mg/ml.

Calcium uptake measurements. Ca^{2+} uptake was measured by either of two assays. One employed a rapid filtration method similar to that of Martonosi and Feretos [10]. The reaction buffer contained 5 mM MOPS, 0.1 M KCl, 4 mM $MgCl_2$, 5 mM oxalate and 0.1 mM $^{45}CaCl_2$ (6500 cpm/nmol Ca^{2+}), pH 7.0. The SR vesicles were preincubated in the reaction mixture for 5 min at 25°, and the reaction was started by the addition of 10 mM ATP in a volume of reaction buffer (readjusted to pH 7.0) producing a final ATP concentration of 4 mM. At various times, aliquots (0.3 to 0.5 ml) were removed, and the SR vesicles were collected by filtration through 0.45 μm pore size type HA Millipore filters (Millipore Corp., Bedford, MA). $^{45}Ca^{2+}$ uptake was determined from both the accumulation of radioactivity on the filters and by loss from the filtrate. The air-dried filters were added to 5 ml of liquid scintillation solution and, after approximately 15 min, they had become transparent and were counted. The filters did not alter the $^{45}Ca^{2+}$ counting efficiency as determined by an external standard.

Calcium uptake was also determined using the metallochromic Ca^{2+} indicator arsenazo III and a method similar to that of Beeler and Gable [11]. The reaction buffer contained 15 mM MOPS, 0.1 M KCl, 2 mM $MgCl_2$, 1 mM ATP, 20 μM $CaCl_2$ and 0.1 mM arsenazo III, pH 7.0. Extravesicular Ca^{2+} concentration was determined by monitoring the change in 700 nm − 655 nm absorbance. Reactions were initiated by addition of 0.1 mg SR protein/ml to the sample cuvette (3.0 ml final volume) which was equipped with a magnetic stirrer. Both cyclopiazonic acid and A23187 were dissolved in dimethyl sulfoxide (DMSO) and added at less than 0.4% (v/v) as DMSO. Controls consisted of addition of similar volumes of DMSO.

Biochemical assays. Protein was determined by the method of Lowry *et al.* [12] using bovine serum albumin as a standard, and inorganic phosphate was assayed by the method of Baginski *et al.* [13] as modified by Ottolenghi [14].

Results and discussion

The uptake of Ca^{2+} by rat SR vesicles in the presence of the Ca^{2+}-precipitating anion oxalate was near linear for the initial 90 sec of the reaction (Fig. 2a). Elimination of SR vesicles, oxalate or substitution of ADP for ATP in the reaction buffer resulted in undetectable levels of Ca^{2+} uptake using this system (data not shown). When cyclopiazonic acid was added to the SR vesicles prior to initiation of the reaction by ATP addition, there was a dose-dependent inhibition of the initial rate (20–90 sec) of Ca^{2+} uptake when compared to controls (Fig. 2a). A log plot of added cyclopiazonic acid versus percent inhibition of the initial rate of Ca^{2+} uptake is shown in Fig. 2a (inset).

The uptake of Ca^{2+} by SR vesicles (0.1 mg protein/ml) and the effect of cyclopiazonic acid as determined by the metallochromic indicator arsenazo III are shown in Fig. 2b. In control SR vesicles, Ca^{2+} uptake was rapid during the first 10 sec of the reaction with a slowing of this rate until approximately 95% of the total was accumulated by 1 min. Cyclopiazonic acid inhibited the steady-state level of total accumulated Ca^{2+} in a dose-dependent manner (Fig. 2b). The onset of this inhibition was rapid occurring within 5 sec after introduction of the SR vesicles to the cyclopiazonic acid containing reaction buffer. When present at levels of less than 25 nmol/mg SR protein, cyclopiazonic acid did not affect Ca^{2+} uptake significantly, using this procedure (data not shown). Addition of the Ca^{2+} ionophore A23187 (15 nmol/mg SR protein) to the treated SR vesicles when reactions were near equilibrium resulted in a rapid return of Ca^{2+} concentrations to levels present before Ca^{2+} uptake was initiated (Fig. 2b). The final Ca^{2+} concentration reached after addition of A23187 was similar between all treatments regardless of accumulated levels prior to the ionophore addition.

Table 1 shows that cyclopiazonic acid also inhibited the Ca^{2+}-dependent ATPase activity of SR vesicles. Both Ca^{2+} uptake and ATPase activity were concurrently determined

Table 1. Cyclopiazonic acid inhibition of Ca^{2+} uptake and Ca^{2+}-ATPase activity of rat skeletal muscle sarcoplasmic reticulum vesicles

	% Inhibition	
CPA (nmol/mg protein)	Ca^{2+} uptake	Ca^{2+}-dependent ATPase activity
25	50	42
50	66	60
100	85	73

The effect of cyclopiazonic acid (CPA) on oxalate-assisted Ca^{2+} uptake and concurrently measured Ca^{2+}-dependent ATPase activity were determined on SR vesicles (0.1 mg protein/ml) during the first 90 sec of incubation. Values are means of four observations. Control Ca^{2+} uptake and Ca^{2+}-dependent ATPase activities were 0.72 ± 0.07 (±SE) μmol Ca^{2+}/mg protein/min and 0.82 ± 0.44 μmol P_i liberated/mg protein/min respectively.

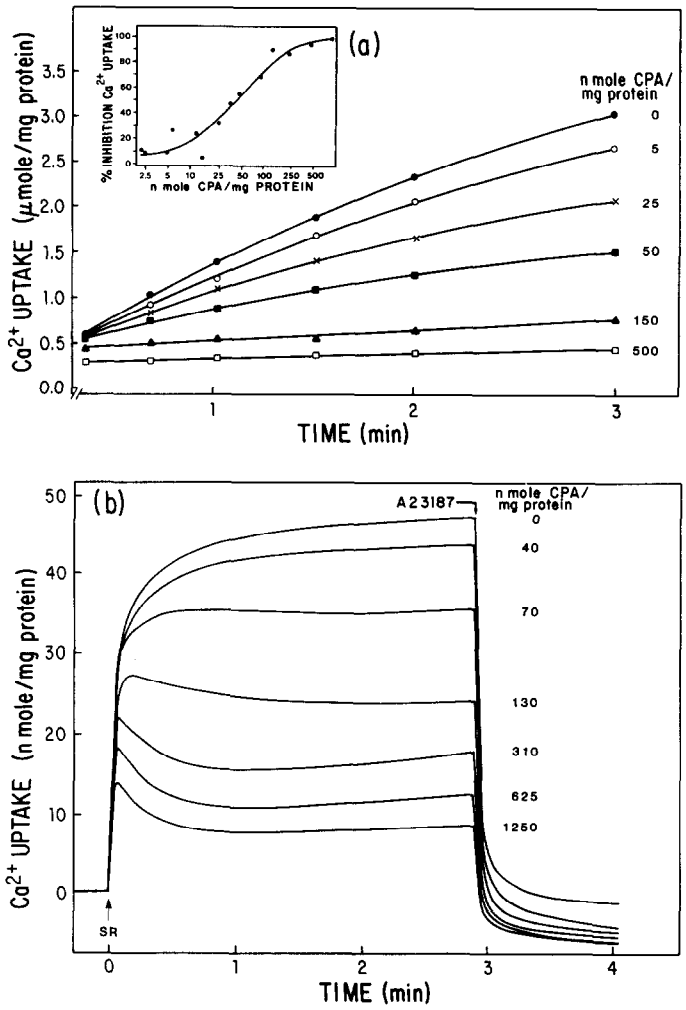

Fig. 2. Cyclopiazonic acid inhibition of Ca^{2+} uptake by rat skeletal muscle sarcoplasmic reticulum vesicles. The effect of cyclopiazonic acid at indicated levels on Ca^{2+} uptake was determined using two separate methods: (a) oxalate-assisted uptake using 0.02 mg SR protein/ml (N = 2–5 observations per point); inset shows the percent inhibition of the initial rate (20–90 sec) of Ca^{2+} uptake vs cyclopiazonic acid concentration, and (b) uptake and release using the metallochromic indicator arsenazo III; 2 μl of a 2 mM solution of the Ca^{2+} ionophore A23187 was added after approximately 3 min of incubation.

on SR vesicles (0.1 mg protein/ml) in the presence of oxalate. Calcium-dependent ATPase activity was determined from total ATPase activity minus the basal activity, that portion present without added Ca^{2+} and in the presence of 1 mM EGTA. Cyclopiazonic acid at 100 nmol/mg protein had no effect on the basal ATPase activity (data not shown).

The results presented here show that cyclopiazonic acid is a potent inhibitor of both Ca^{2+} uptake and the Ca^{2+}-dependent ATPase activity of SR vesicles prepared from rat skeletal muscle. It is possible that this inhibitory action may be involved in cyclopiazonic acid toxicity. Concentrations of cyclopiazonic acid reported in the skeletal muscle of rats and chickens dosed with this compound [4, *] are of the same order of magnitude as those producing inhibition in the present study.

* W. P. Norred, J. K. Porter, R. J. Cole and J. W. Dorner, manuscript submitted for publication.

† To whom correspondence should be addressed.

U.S. Department of Agriculture
Agricultural Research Service
Richard B. Russell Agricultural Research Center
Athens, GA 30613; and

DOUGLAS E. GOEGER
RONALD T. RILEY†

U.S. Department of Agriculture
Agricultural Research Service
National Peanut Research Laboratory
Dawson, GA 31742, U.S.A.

JOE W. DORNER
RICHARD J. COLE

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Time- and dose-dependent inhibition of erythrocyte glutathione peroxidase by cisplatin*

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Cisplatin-induced anemia is a well-known side effect [1] that develops in 9–40% of patients treated with this drug [2]. Acute hemolytic anemia has also been reported, although more rarely [3, 4]. Existence of a direct myelotoxic effect is an unsatisfactory hypothesis because leukopenia and thrombocytopenia are rare [2]. Direct toxicity of CDDP on red blood cells (RBS) has also been suggested. CDDP has already been shown to interact with glutathione (GSH) [5]. Because GSH and its related enzymatic system are a significant source of cellular detoxification [6], especially for RBC, we investigated the biochemical effects of CDDP on RBC enzymatic pathways.

A time and pharmacologically compatible dose study was thus performed *in vitro*, in triplicate using blood from three healthy volunteers (males aged 28, 35 and 38 yr). Study parameters were as follows: GSH [7], oxidized glutathione (GSSG) [7], 6-phosphogluconic dehydrogenase (EC 1.1.1.44) [8], hexokinase isomerase (EC 5.3.1.9) [8], glutathione peroxidase (EC 1.11.1.9, GSH Px) [9] and glutathione reductase (EC 1.6.4.2) [10]. Whole blood was incubated at 37° under slight agitation. Five ml blood samples were treated with four different doses (0, 1, 5 and 10 μg/ml). After appropriate intervals (0, 30 min, 1, 3, 6, 24 and 48 hr), the reaction was stopped by immersion in an ice bath (4°); this was followed by separation of the buffy coat, washing with one volume of saline solution, centrifugation at 4°, and rapid analysis of 6-phosphogluconic dehydrogenase, glucose-6-phosphate dehydrogenase, hexokinase, pyruvate kinase and phosphoglucose isomerase. An aliquot of erythrocytes (1 ml) was also quickly stored at −20° until analysis of GSH, GSSG, GHS Px and GSH reductase. Data were examined for dose and incubation time effects by two-way ANOVA analysis with repeated measurements.

As shown in Fig. 1, GSH Px was inhibited as a function of both time and the CDDP dose. A parallel inhibition occurred in GSH consumption and GSSG synthesis. For these parameters, ANOVA analysis revealed a highly significant effect, without interaction, for both the CDDP dose and the length of the incubation. In contrast, no significant modifications were observed in the other enzyme activities tested (Table 1).

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As shown by our data, CDDP exposure at concentrations compatible with existing pharmacokinetic information (1–10 μg/ml) [11–13] can significantly deplete RBC GSH Px activity. As GSH Px generates GSSG from GSH, concomitant inhibition of GSH consumption and GSSG synthesis is compatible with GSH Px depletion. These effects are strongly dose- and time-dependent. They are specific

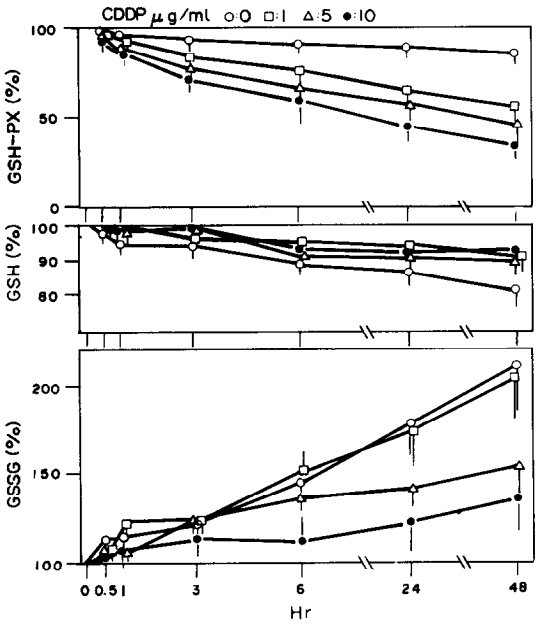

Fig. 1. Effect of incubation time and CDDP dose on GSH Px activity, GSH consumption and GSSG synthesis. Results are expressed as the percentage of values at t = 0. Two-way ANOVA analysis with repeated measurements gave the following F values for the effect of incubation time (F^6_{42}), dose (F^2_{42}) and interaction (F^{12}_{42}). For GSH Px activity: $F^6_{42} = 12.6$ ($P < 0.001$), $F^2_{42} = 10.7$ ($P < 0.001$), $F^{12}_{42} = 0.39$ NS. For GSH consumption: $F^6_{42} = 43.1$ ($P < 0.001$), $F^2_{42} = 7.79$ ($P < 0.01$), $F^{12}_{42} = 0.99$ NS. For GSSG production: $F^6_{42} = 3.67$ ($P < 0.01$), $F^2_{42} = 6.4$ ($P < 0.01$), $F^{12}_{42} = 0.89$ NS.