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IMPAIRED ABSORPTION OF CHLORPROMAZINE IN RATS GIVEN TRIHEXYPHENIDYL

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1 The absorption and tissue distribution of orally administered [^{14}C]-chlorpromazine (CPZ) were compared in trihexyphenidyl (THP; Artane)-treated and control rats.

2 Total radioactivity (CPZ) in the plasma and brain of rats treated with THP was significantly lower whereas total radioactivity in the stomach was significantly higher than in rats not previously treated with THP.

3 Gastric emptying in rats treated with THP was significantly delayed as measured by gastric clearance of a marker, [^{14}C]-polyethylene glycol.

4 Transport of [^{14}C]-CPZ in everted sacs was not affected by treatment with THP.

5 Metabolism of [^{14}C]-CPZ by liver homogenates was not affected by treatment with THP.

6 The relationship of delayed gastric emptying in THP-treated rats and their lower plasma and brain levels of [^{14}C]-CPZ after oral administration is discussed.

Introduction

Trihexyphenidyl (THP; Artane), a parasympatholytic drug, has been used clinically in the symptomatic treatment of Parkinson's disease since 1949. Because of its antiparkinsonian activity, it has been used with neuroleptic drugs routinely or prophylactically in psychiatry to prevent the occurrence of extrapyramidal symptoms. Observations by Singh and colleagues (Singh & Smith, 1973; Singh & Kay, 1974; Singh & Kay, 1975) indicated reversal of the therapeutic effects achieved with neuroleptics when parasympatholytics were added to the therapeutic regimen. Concurrently, our double-blind studies in psychiatric patients (Rivera-Calimlim, Castañeda & Lasagna, 1973; Rivera-Calimlim, Nasrallah, Strauss & Lasagna, 1975) showed that trihexyphenidyl consistently lowers plasma levels of chlorpromazine (CPZ). The present study was intended to reproduce this trihexyphenidyl-CPZ interaction in rats and to investigate the site of this interaction.

Methods

Chronic treatment

Groups of eight male Sprague-Dawley rats (160–180 g), housed in individual cages and maintained on regular laboratory diet and water, were treated daily with trihexyphenidyl, administered orally, for two weeks. Trihexyphenidyl, (THP; Artane, Lederle Laboratories, Pearl River, New York) as a dose of 50 mg/kg was dissolved in water and given orally in a 1 ml solution once a day through a metal gastric tube. Matched controls received an equivalent volume of water.

At the end of the pretreatment period, both THP-treated and control rats were given an oral dose of [^{14}C]-CPZ (400 μg, 5 μCi) after a 12 h fast. [^{14}C]-CPZ hydrochloride (6.75 mCi/mmol) labelled in the ring was obtained from Applied Science Laboratories, Inc., State College, Pennsylvania. Two hours after the [^{14}C]-CPZ dose, the animals were anaesthetized with ether, an abdominal incision was made, and 5–10 ml of blood was drawn from the inferior vena cava into a tube containing heparin. The stomach was dissected out and its contents were drained into a tube; it was then cut open and washed three times with 0.01 N HCl, and the washings were collected. The stomach tissue was weighed and homogenized in 5 ml of 0.1 N HCl with a tissue homogenizer (Brinkman Polytron, Switzerland).

The brain was dissected out and washed three times with 0.01 N HCl and the washings were discarded. The brain was homogenized in 5 ml of 0.1 N HCl. The blood was centrifuged and the plasma was separated. Total radioactivity was assayed by adding aliquots of plasma (0.2 ml), stomach washings (0.5 ml), and stomach and brain homogenates (0.2 ml) solubilized in

Nuclear Chicago Solubilizer (NCS) to 10 ml of Triton X-toluene scintillation liquid and counting in a Packard Tri-Carb Scintillation Spectrometer. Counting efficiency for ^{14}C was 85–88%. All values of radioactivity were corrected for quenching and background.

Acute treatment

The distribution of radioactivity in the plasma, brain, and stomach 2 h after oral administration of [^{14}C]-CPZ in rats pretreated with one dose of THP (50 mg/kg) was studied following the procedure described above for chronic treatment. The THP was administered orally 1 h before an oral dose of [^{14}C]-CPZ. Matched controls were given water orally instead of THP before the dose of [^{14}C]-CPZ.

Measurement of gastric emptying

Male Sprague-Dawley rats were treated orally with THP (50 mg/kg) for one week, with matched controls receiving daily administration of water orally. After a 12 h fast, on the day of the experiment, the THP-treated and control rats were given [^{14}C]-labelled polyethylene glycol ([^{14}C]-PEG; 0.2 μCi) orally. Two hours later the rats were decapitated, and the stomach was dissected out, opened, and drained. The stomach cavity was washed with 0.01 N HCl three times, and the washings were collected for assay of radioactivity.

Effect of trihexyphenidyl on intestinal transport

The effect of THP on the transport of [^{14}C]-CPZ in everted sacs was studied under three different conditions as follows: (1) Effect of trihexyphenidyl when added directly to the incubation medium at the same time as [^{14}C]-CPZ was added. (2) Effect of 15 min preincubation of the everted sacs in THP solution, before testing the transport of [^{14}C]-CPZ. (3) Transport of [^{14}C]-CPZ by the everted sacs prepared from rats that were treated chronically with THP for 2 weeks.

General procedure of the everted sac experiment

Jejunal everted sacs were prepared from rats fasted for 12 h, by the method of Wilson & Wiseman (1954). The everted sac was filled with 0.5 ml of modified Krebs-bicarbonate buffer (pH 7.4) (Rivera-Calimlim, 1972) and securely closed with a ligature. The sacs, with appropriate treatment and substrate ([^{14}C]-CPZ) were incubated separately in 25 ml of Krebs-bicarbonate buffer (pH 7) for 30 min under 95% O_2 and 5% CO_2 at 100 oscillations per min in a Dubnoff metabolic shaking incubator. At the end of the incubation period the sacs were quickly removed and rinsed in 5–10 ml of ice-cold buffer. The serosal fluid was collected and measured, and the tissue was placed in a pre-weighed scintillation vial, dried in an oven at 105°C overnight and weighed. The tissues were solubilized in 1.5 ml NCS tissue solubilizer and the radioactivity was assayed in 10 ml of Triton X-100 toluene scintillation liquid in a Packard Liquid Scintillation Spectrometer. Counting efficiency was 88–90%. All counts were corrected for background and quenching.

The concentration of [^{14}C]-CPZ in the incubation medium was 1 mM (2 μCi per sac). The concentration of THP in both acute *in vitro* addition and preincubation was 1 mM. THP was not added to the incubation medium of the everted sacs prepared from THP chronically treated rats. The chronically treated rats were given oral THP (50 mg/kg) daily for 2 weeks; the last dose was given 1 h before they were killed.

Effect of trihexyphenidyl on the liver metabolism of chlorpromazine

Male Sprague-Dawley rats (120–150 g) were treated with oral THP daily, 50 mg/kg, for 1 week, with matched controls. At the end of the course of treatment the rats were fasted for 18 h and killed by decapitation, and the liver was dissected out. Approximately 1.5 g was homogenized in 4 volumes (4 times tissue weight) of 1.15% KCl–0.1M phosphate buffer (pH 7.4), the preparation being kept in ice throughout the procedure.

A sample of homogenate (0.5 ml) was added to 50 ml polystyrene tubes containing 1 ml of 0.1 M phosphate buffer (pH 7.4), 90 μmol of $MgCl_2$, 0.4 μmol of NADP, 15 nmol of glucose-6-phosphate, 1 i.u. glucose-6-phosphate dehydrogenase, and 0.5 μmol of [^{14}C]-CPZ (0.2 μCi). The total volume of incubation was 2 ml. The tubes were incubated at 37°C in a Dubnoff metabolic shaking incubator for 10 min at 100 oscillations/minute. After incubation, the chlorpromazine metabolites were separated from the parent compound by the solvent extraction technique of Curry, Derr & Maling (1970), with minor modifications. Samples (1 ml) were taken from the final heptane layers (after extraction of the heptane first with NaOH and then with sodium acetate buffer, pH 4.7) for assays of radioactivity, which represents unchanged chlorpromazine. Appropriate control samples were run simultaneously to determine the recovery of chlorpromazine, added to tissue homogenates, through the whole of the extraction procedure. This was found to be $57.7 \pm 0.6\%$. All experimental values were, therefore, corrected for this recovery.

Results

Table 1 shows that rats treated chronically with THP for 2 weeks achieved significantly lower plasma levels

(20% of control) and lower brain levels (10% of control) of radioactivity from [^{14}C]-CPZ administered orally 2 h before killing, with significant retention of [^{14}C]-CPZ in the stomach contents of THP-treated rats. The radioactivity of the stomach contents was increased eight-fold in the THP-treated rats when compared to that of controls; radioactivity of the stomach wall in the treated rats was doubled. The same distribution pattern of oral [^{14}C]-CPZ was observed in rats that received only one dose of THP, although the changes observed were less marked than in the rats chronically treated with THP (Table 2). To test whether the stomach retention was due to the dose of THP, which inhibited gastric emptying *in vivo*, did not inhibit mucosal transport of [^{14}C]-CPZ in everted sacs prepared from the same rat.

Table 5 shows that the metabolism of [^{14}C]-CPZ was not increased after incubation with liver homogenates prepared from rats treated with THP for 7 days.

Discussion

The lowering effect of trihexyphenidyl on plasma CPZ levels that has been observed in psychiatric patients

Table 1 Distribution of orally administered [^{14}C]-chlorpromazine ([^{14}C]-CPZ) in rats chronically treated with trihexyphenidyl

Sample	*Controls* (n=8)	*Treated* (n=8)	P
Plasma (d min^{-1} ml^{-1})	2940.3±309	613.4±107	<0.001
Brain (%)*	0.068±0.008	0.007±0.005	<0.01
Stomach contents (%)*	3.08±0.76	26.85±5.87	<0.01
Stomach wall (%)*	3.24±0.75	7.63±0.31	<0.01

Values are expressed as mean±s.e. mean; n=number of rats. Significance of the difference was tested by the Student t test. Rats pretreated with oral trihexyphenidyl (50 mg/kg) and their controls were killed 2 h after an oral dose of [^{14}C]-CPZ (5 μCi). * Percent of the total dose given.

Table 2 Distribution of orally administered [^{14}C]-chlorpromazine ([^{14}C]-CPZ) in rats pretreated with one oral dose of trihexyphenidyl

Sample	*Controls* (n=8)	*Treated* (n=8)	P
Plasma (d min^{-1} ml^{-1})	3120±293	920±124	<0.01
Brain (%)*	0.078±0.01	0.034±0.007	<0.01
Stomach contents (%)*	3.52±0.34	14.85±3.87	<0.01
Stomach tissue (%)*	2.37±0.20	9.33±1.52	<0.01

Values are means±s.e. mean; n=number of rats. Conditions are the same as for Table 1. * Percent of the total dose.

inhibition of gastric emptying or to gastric secretion of absorbed CPZ, gastric emptying was measured by the use of a marker, [^{14}C]-PEG, a substance which is neither adsorbed, metabolized or secreted. Results are shown in Table 3. The delay in gastric clearance of PEG indicates slow gastric emptying. Even 1 h after oral administration of [^{14}C]-PEG, 41–63% of the administered dose was recovered from the stomach of the rats pretreated with THP, but none was recovered from that of control rats.

The possibility that THP also affects direct intestinal mucosal transport and liver metabolism of CPZ was studied. Table 4 shows that the mucosal transport of [^{14}C]-CPZ in the everted sacs exposed to THP in three different ways was not significantly different from that in matched controls. The 50 mg/kg

Table 3 Gastric emptying as measured by [^{14}C]-polyethylene glycol ([^{14}C]-PEG)

Dose of THP (mg/kg)	*% [^{14}C]-PEG in stomach wash*	
	Control	*Treated*
50	0.1±0.06	41.3±1.3
100	0.1±0.06	63.3±17.3

Values are expressed as mean (±s.e. mean) percentage of recovery of total dose of [^{14}C]-PEG given orally, one hour after administration. Trihexyphenidyl (THP) was given orally one hour before [^{14}C]-PEG. The difference between control and treated on both doses was statistically significant (P<0.01). The difference between the treated groups given different doses was not significant.

Table 4 Effect of trihexyphenidyl (THP) on transport of [^{14}C]-chlorpromazine ([^{14}C]-CPZ) in everted sacs

Experiment and incubation time	Recovery of CPZ in: Serosal fluid (μmol/g) Control	Serosal fluid (μmol/g) Treated	Gut tissue (μmol/g) Control	Gut tissue (μmol/g) Treated
Addition of THP to medium				
15 min	0.63 ± 0.18	0.49 ± 0.07	27.6 ± 2.6	28.6 ± 4.0
30 min	0.62 ± 0.13	0.61 ± 0.21	42.2 ± 2.64	38.0 ± 1.83
Everted sacs prepared from chronically treated rats				
60 min	2.7 ± 0.2	2.9 ± 0.5	44.1 ± 1.9	49.2 ± 2.2
Everted sacs pre-incubated in THP for 15 min				
15 min	0.38 ± 0.05	0.37 ± 0.04	25.0 ± 1.7	26.8 ± 1.9
30 min	0.86 ± 0.19	0.94 ± 0.07	40.66 ± 2.4	40.57 ± 4.1

Values are expressed as mean ± s.e. mean; $n = 6$. Significance of the difference between TPH-treated and controls was tested by Student's t test and all P values were greater than 0.5.

Table 5 Effect of trihexyphenidyl on [^{14}C]-chlorpromazine ([^{14}C]-CPZ) metabolism by rat liver *in vitro*

Source	μg CPZ metabolized per g liver tissue
Treated ($n = 8$)	574.8 ± 43.0
Control ($n = 8$)	512.1 ± 32.7

Values are the mean ± s.e. mean; n = number of rats. Significance of the difference was tested by the Student t test; $P > 0.5$. Samples were run in duplicate.

given both drugs was shown to be reproducible in rats in the present study. At the time when low plasma levels of CPZ were observed in THP-treated rats, the brain levels were also reduced, whereas in the stomach the CPZ levels were significantly higher than in control rats. This pattern of tissue distribution of CPZ (Table 1) in rats treated with THP was observed in both the chronic and the acute experiments and suggested that the lower plasma levels of CPZ in treated rats may be due to inhibition of gastric emptying resulting from the antiacetylcholine property of THP. Rivera-Calimlim, Dujovne, Morgan, Lasagna & Bianchine (1971) have observed that absorption of certain drugs is affected by inhibition of gastric emptying, caused either by increase in gastric acidity or by antiacetylcholine agents such as imipramine (Morgan, Nathan & Rivera-Calimlim, 1972; Morgan, Nathan, Rivera-Calimlim & Trabert, 1975) and Adjepon-Yamah, Scott & Prescott (1973) have reported a striking delay in the absorption of oral lignocaine in patients treated with atropine prior to laparoscopy. Similarly, Consolo, Morselli, Zaccala & Garratini (1970) have shown that desmethyl imipramine delays the absorption of phenylbutazone. Nimmo, Heading, Tothill & Prescott (1973) demonstrated the inhibitory effect of propantheline, an antiacetylcholine agent, on absorption of paracetamol.

The effect of THP on gastric emptying was, therefore, studied by the use of radioactive PEG as a marker and the results showed conclusively that THP significantly delayed the gastric emptying. The other possible mechanisms whereby THP can lower plasma levels of CPZ are a direct inhibition of THP of intestinal absorption or a stimulation by THP of the liver metabolism of CPZ. THP has no effect on intestinal tissue uptake and mucosal transport of CPZ in everted sacs. Likewise, the *in vitro* metabolism of CPZ by liver homogenates from rats treated chronically with THP did not change significantly when compared with the controls. It can, therefore, be concluded from this study that the lower plasma levels of CPZ observed in rats when CPZ is administered with THP is due to inhibition of gastric emptying, and this may also be the mechanism involved in man.

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