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TISSUE LEVELS OF MESCALINE IN MICE: INFLUENCE OF CHLORPROMAZINE ON REPEATED ADMINISTRATION OF MESCALINE[1]

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The present study indicates that administration of CPZ (15 mg/kg) to mice 30 min prior to or 45 min after a tracer dose of mescaline, caused marked retention of the hallucinogen in the brain and other tissues examined. At 3 hr, the levels of mescaline in the brain, eye, liver and plasma were 3–6 times more than those in corresponding tissues of control mice. A second dose of mescaline injected 3 hr after the first dose, further enhanced the levels of mescaline in all tissues of CPZ-treated mice. The effect of CPZ appears to be due to the blockade of the removal of mescaline from various tissues since the initial entry of the hallucinogen remained unaffected.

Chlorpromazine Retention Mescaline Repeated administration

1. Introduction

Chlorpromazine (CPZ) evokes a multitude of effects on various metabolic pathways. There is evidence that it affects the membrane permeability to amines at various cellular and subcellular sites (Dengler et al., 1961; Carlsson et al., 1963; Pletscher et al., 1965). A few studies have shown a marked prolongation of the disappearance of injected melatonin (Wurtman and Axelrod, 1966) and amphetamine (Sulser and Dingell, 1968; Borella et al., 1969; Lemberger et al., 1970) in rats pretreated with CPZ (15–20 mg/kg). Recently we have demonstrated that administration of CPZ (15 mg/kg) 30 min prior to or 45 min after mescaline caused a marked retention of the hallucinogen in various tissues of mice (Shah et al., 1972, 1973). Present study reports the effect of CPZ on the accumulation of mescaline in several tissues of mice when latter was administered twice 30 min and 3.5 hr after the former drug.

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2. Materials and methods

2.1. Materials

8-^{14}C-mescaline·hydrochloride (Sp. act. 4.53 mCi/mmol) was purchased from New England Nuclear Corporation (Boston, Mass.). The purity was ascertained in our laboratory by paper and column chromatography and found to be approximately 97%. CPZ · hydrochloride was received as a gift from Smith, Kline and French Laboratories (Philadelphia, Pa.).

2.2. Procedure on animals

2.2.1. Single dose

Male albino mice of Swiss–Webster strain (30–33 g) were used. 2 groups of 6 mice each were injected i.p. with either saline or CPZ (15 mg/kg) in 0.5 ml. 30 min later, they were injected a tracer dose of ^{14}C-mescaline (2 μCi) and sacrificed 1, 2, 3 and 6 hr from the time of mescaline administration.

2.2.2. Double dose

4 additional groups of 4–8 mice each received saline or CPZ (15 mg/kg) followed by ^{14}C-mescaline (2

μCi) 30 min later. At the end of 3 hr after the first dose, a second dose of ^{14}C-mescaline (2 μCi) was given and the animals were sacrificed by decapitation 4 and 6 hr after the first dose of mescaline. In a few experiments, mice received a first dose of mescaline (2 μCi) and 45 min later were injected with CPZ (15 mg/kg). At the end of 3 hr after the first injection of mescaline, a second dose of hallucinogen (2 μCi) was administered and the mice were sacrificed 6 hr from the time the first dose of mescaline was given.

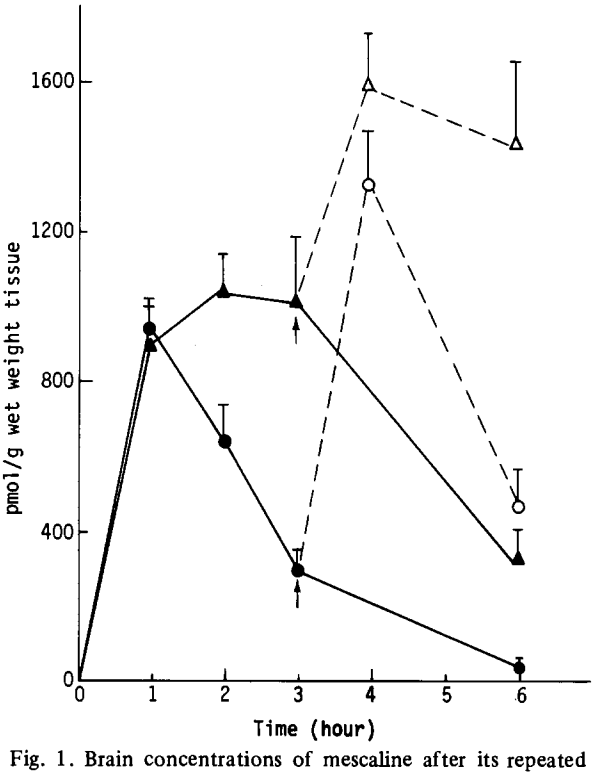

Fig. 1. Brain concentrations of mescaline after its repeated administration at 3 hr interval. Solid circles and solid triangles connected by solid lines: mescaline levels in non-CPZ- and CPZ-treated mice receiving single dose of mescaline (2 μCi). Open circles and open triangles connected by broken lines: mescaline levels in non-CPZ- and CPZ-treated mice receiving second injection of mescaline (2 μCi) 3 hr after the first dose (shown by arrow). In a single dose experiment, the 2, 3 and 6 hr, and in a double dose experiment, 6 hr brain mescaline levels are significantly higher in CPZ-pretreated mice as compared to those in control mice ($p<0.001$); the 4 hr levels (double dose studies) are significantly different ($p<0.05$).

2.3. Extraction and counting of radioactivity

Plasma was separated from oxalated blood; the tissues were promptly removed, frozen on dry ice and stored at −20°C until assays were performed. The radioactivity in tissues was extracted in 0.4 N perchloric acid (Shah et al., 1968). After adjusting the pH to 5.8 with 5 N potassium carbonate, the extracts were centrifuged in cold to sediment potassium perchlorate. Aliquots of 0.5 ml were added to 10 ml scintillation fluid (Bray, 1960) for the assay of total radioactivity. The remaining portions were allowed to pass over columns containing Dowex 50-WX$_4$ for the separation of unchanged mescaline according to the procedure outlined by Charalampous et al. (1966) with some modifications (Shah and Himwich, 1971). Radioactivity was counted in a Nuclear Chicago Mark II liquid scintillation spectrometer using ^{133}Ba as an external standard. The efficiency of the counting system ranged between 86 and 90%. The overall recovery of radioactivity through the isolation procedure was between 72 and 80%. All data have been corrected for counting efficiency and for recovery.

Values are averages of 4 to 8 separate experiments ± S.D. of the mean and are reported as pmol per g of wet weight tissue or per ml. The statistical significance of differences between mean values was determined with a 2-tailed Student's *t*-test.

3. Results

3.1. Brain mescaline levels

3.1.1. Single dose

Determination of brain mescaline levels was carried out at different times after administration of ^{14}C-mescaline (fig. 1). In saline-treated mice the mescaline contents were highest at 1 hr post injection (943 ± 81 pmol/g; solid circle). The disappearance was rapid; at 2, 3 and 6 hr the levels were 628 ± 94, 289 ± 75 and 40 ± 16 pmol/g respectively. In CPZ-pretreated mice the levels of mescaline at 1 hr were almost identical to those in saline-treated controls (solid triangle); compared with controls, 2, 3 and 6 hr brain levels were statistically significantly higher in CPZ-treated animals. It is noteworthy, that 6 hr after the administration of mescaline, the levels of the drug in the brain of CPZ-treated mice were approximately the same as those in the brain of control mice.

3.1.2. Double dose

In saline-treated controls, the 4 and 6 hr brain levels of mescaline were 1317 ± 145 and 460 ± 108 pmol/g respectively (open circle). Close examination of 4 hr values would indicate a cumulative effect of 3 and 1 hr levels after single administration. Thus, in a single dose study the 3 hr level was 289 pmol/g; following a second dose, the levels of mescaline were raised by additional 1028 pmol, a level that is usually attained 1 hr after a single injection of 2 μCi of mescaline. The levels of mescaline in brains of CPZ-treated animals at 4 and 6 hr were 1570 ± 148 and 1428 ± 205 pmol/g respectively (open triangle). A second dose of mescaline had raised the level by about 560 pmol over the levels obtained at the end of 3 hr after the first injection.

3.1.3. Liver, eye and plasma mescaline levels

In a single dose experiment the mescaline contents in CPZ-treated mice at 3 hr were approximately 5–6 times and at 6 hr were 3–5 times more than in corresponding tissues of controls (table 1). In a double dose experiment, the mescaline levels in CPZ-treated animals at 4 hr were doubled but at 6 hr were 4–7 times greater than in controls.

3.1.4. Heart, kidney, lung and spleen mescaline levels

Animals were injected with saline or CPZ (15 mg/kg); ^{14}C-mescaline, 2 μCi each, was injected i.p. 30 min and 3.5 hr after CPZ. The animals were sacrificed 6 hr after the first injection of mescaline. From 7 to 14 times more mescaline was accumulated in kidney, lung, heart and spleen of CPZ-pretreated mice when compared with the levels in corresponding tissues of control animals (table 2).

3.1.5. Effect of post treatment with CPZ

Mice were given mescaline (2 μCi) followed 45 min later with CPZ (15 mg/kg). A second dose of mescaline (2 μCi) was administered 3 hr from the time of first mescaline injection and the animals were sacrificed 6 hr from the beginning of the experiment. The levels of mescaline in the brain, eye, liver and plasma (table 3) were not markedly different from those in the corresponding tissues of mice receiving CPZ (15 mg/kg) prior to two injections of mescaline as shown in fig. 1 and table 1.

Table 1

Effect of CPZ on the disappearance of ^{14}C-mescaline following single or repeated administration of mescaline.

Mice were injected either saline or CPZ (15 mg/kg) 30 min before 1st dose of mescaline (2 μCi). An additional dose of mescaline (2 μCi) was administered 3 hr after 1st dose and animals were sacrificed 4 and 6 hr from the time of 1st mescaline injection. In single dose experiments, the levels of mescaline were measured 3 and 6 hr after administration of hallucinogen. Each value is an average of at least 4–8 separate experiments ± S.D. p = significance of differences between mean values.

	Unchanged ^{14}C-mescaline (pmol/g or /ml)			
	Single dose		Double dose	
	3 hr*	6 hr*	4 hr*	6 hr*
Liver				
Saline	1165 ± 389	246 ± 54	16860 ± 4703	2723 ± 925
CPZ	5431 ± 992	826 ± 158	28142 ± 3407	10671 ± 4351
p	< 0.001	< 0.001	< 0.001	< 0.001
Eye				
Saline	306 ± 76	170 ± 10	3524 ± 787	649 ± 344
CPZ	1901 ± 148	855 ± 259	6582 ± 1397	4466 ± 1921
p	< 0.001	< 0.001	< 0.001	< 0.001
Plasma				
Saline	311 ± 111	122 ± 33	2770 ± 537	596 ± 209
CPZ	1532 ± 300	352 ± 97	5978 ± 1502	4106 ± 2111
p	< 0.001	< 0.001	< 0.001	< 0.001

* Time of sacrifice.

Table 2
Effect of CPZ on the disappearance of ^{14}C-mescaline after repeated administration of mescaline.
Animals were injected with CPZ (15 mg/kg); ^{14}C-mescaline 2 μci each, was injected i.p. 30 min and 3.5 hr after CPZ. The animals were sacrificed 6 hr after the first injection of mescaline. Each value is an average of 8 (control) and 4 (CPZ) separate experiments ± S.D.

Tissue	Unchanged ^{14}C-mescaline (pmol/g of net weight tissue)		% Change from control
	Control (saline)	CPZ	
Heart	641±149	7274±1121*	1134
Kidney	2596±748	17,778±1005*	684
Lung	2012±393	19,667±1685*	977
Spleen	1078±182	14,621± 632*	1356

* Significantly different from the corresponding control levels; $p < 0.001$.

Table 3
Levels of mescaline in tissues of mice injected with mescaline (2 μCi) followed 45 min later by CPZ (15 mg/kg) and 3 hr later by second dose of mescaline (2 μCi). Animals were sacrificed 6 hr after the first injection of mescaline. Each value is an average of 4 separate experiments ± S.D.

Tissue	Unchanged ^{14}C-mescaline (pmol/g or /ml)
Brain	1282 ± 227
Liver	7979 ± 1594
Eye	3890 ± 1007
Plasma	3638 ± 723

4. Discussion

The aim of this study is to observe the effect of a single dose of CPZ on the disposition of mescaline injected twice at an interval of 3 hr. Our results with single dose experiments indicate that CPZ does not affect the initial accumulation of mescaline in the brain tissue since 1 hr levels were almost identical to those in the brains of saline-treated animals. The data, however, suggest that in CPZ-pretreated animals, the disappearance of mescaline from the brain took place in two phases: a phase one, between 1 and 3 hr and a phase two between 3 and 6 hr. The time interval in phase one could be described a period of retention during which the rate of disappearance is practically zero since 3 hr after the mescaline injection, the brain mescaline contents are almost identical to the peak levels measured in CPZ-pretreated as well as control brains. The time interval in phase two, on the other hand, could be described a period of removal; thus, the slope of the disappearance curve between 3 and 6 hr is steeper for the CPZ group and approximates the rate of phase one of the control group. A possible explanation may be that CPZ can stabilize and expand cell membrane as suggested by Seeman (1966), creating additional binding sites with retention of more mescaline (Denber, 1967). The rapid rate of elimination of mescaline from the brain of CPZ-treated mice between 3 and 6 hr (phase two) could be due to release of mescaline from the binding sites. In a single dose experiment, the subcellular distribution studies revealed an increased binding of mescaline to various particles of brain homogenates obtained from CPZ-treated mice (Shah, 1974; in preparation) when compared with that of controls (Shah, 1971) sacrificed 3 hr after mescaline injection. Further studies have shown a decline in binding of mescaline at 6 hr in CPZ-pretreated mice.

A point of considerable interest was the levels of mescaline in the brain of control and CPZ-pretreated mice in a double dose experiment; in the controls, a second injection of mescaline was associated with a large increase (fig. 1; 4 hr level) and a rapid fall (6 hr level) in mescaline concentration. On the other hand, when the CPZ-pretreated mice were challenged with a second dose of mescaline, the CPZ effect was still apparent, that is, the rate of decline of mescaline was again decreased. In recently published data from our laboratory, we have demonstrated that a single injection of CPZ (15 mg/kg) 30 min, 4 and 16 hr prior to ^{14}C-mescaline administration caused a prolongation of the disappearance of latter from the brain and liver of mice sacrificed 3 hr after the hallucinogen (Shah and Gibson, 1973). In other words, CPZ-induced effect on mescaline retention could be seen for as long as 16 hr. The mechanism by which freshly injected mescaline accumulates in tissues of mice pretreated with CPZ is a matter of speculation. One explanation may be that CPZ-induced cell membrane expansion with the additional binding sites as suggested by Seeman (1966) could be still active and hence responsible for retention of repeatedly injected

mescaline. At present we are exploring this possibility in our laboratory.

It is interesting that CPZ-induced increased tissue levels of mescaline are unrelated to metabolic conversion of mescaline to its principal deaminated metabolite, 3,4,5-trimethoxyphenylacetic acid; in fact, the levels of metabolite in tissues of CPZ-treated mice were somewhat higher than those in corresponding tissues of control group (Shah et al., 1973). In vitro studies also revealed that CPZ has no effect on the oxidative deamination of mescaline by mouse liver homogenate (Shah and Gibson, unpublished data) and by mouse brain homogenate (Seiler and Demisch, 1971). When measured in vitro, CPZ does not affect the monoamine oxidase activity (Ehringer et al., 1960; Johnson et al., 1971). A possibility whether a non-specific liver microsomal enzyme is involved in the metabolism of mescaline and also whether such a metabolism is influenced by CPZ was investigated. Utilizing mouse liver microsomal preparation, we were unable to observe any significant metabolic degradation of mescaline and indeed, CPZ was without any effect. These data strongly emphasize that CPZ-induced mescaline changes are altogether independent of its action on metabolic conversion of mescaline.

CPZ evokes a multitude of effects on various metabolic pathways. For example, an interference with the energy producing processes in skeletal muscle by CPZ has been demonstrated by Peterson et al. (1966). Phenothiazines are also known to influence membrane transport mechanisms (Judah and Ahmed, 1963). CPZ has been shown to affect membrane permeability to amines at various cellular and subcellular sites (Dengler et al., 1961; Stacey, 1961; Carlsson et al., 1963; Pletscher et al., 1965; Shah, 1972). There is evidence that phenothiazine activity may strengthen the membrane against osmotic and physical shock and may also incorporate electrostatic and change transfer properties into membrane surface (Teller, 1964; Seeman, 1966). In recent years many studies have suggested that changes in membrane permeability induced by CPZ may be the basis for many of its reported actions. Meltzer (1971) has suggested that CPZ could interfere with the metabolic processes necessary for the maintenance of cell membrane integrity and permeability. Recently we have reported that the CPZ-induced mescaline retention was unrelated to the hypothermic response of the phenothiazine compound (Shah and Neely, 1973). These observations together with those reported above on the lack of effect of CPZ on the metabolism of mescaline strongly suggest that CPZ might be affecting the tissue disappearance of mescaline by some other mechanisms. CPZ-induced increased binding of mescaline to subcellular particles (see above) and the well-known action of phenothiazines on membrane permeability would suggest that the observed effect of CPZ may be at membrane level. It is also probable that CPZ might interfere with the urinary excretion of mescaline since CPZ-pretreated mice excreted decreased amount of mescaline in the urine (Shah et al., 1973). We are currently examining these various possibilities.

The observed effect of CPZ does not seem to be limited to brain tissue. A marked accumulation of mescaline in the peripheral organs following a second dose suggests that CPZ does not affect the influx of mescaline. Our data indicate that treatment with CPZ would lead to marked and prolonged elevation of mescaline if a second dose is repeated before previously accumulated mescaline levels had subsided.

Quite frequently CPZ is employed clinically in the treatment of toxicity of LSD, amphetamine, mescaline and other hallucinogens (Denber and Merlis, 1956; Clark and Bliss, 1957; Espelin and Done, 1968). Recently, however, disturbing effects of CPZ on adversed LSD and amphetamine reactions have been reported in humans (Silverman, 1970). In some of our experiments, CPZ was administered 45 min after the first dose of mescaline. This situation is analogous to a clinical condition where the manifestations of mescaline toxicity or psychosis are already present and an antipsychotic agent, such as CPZ, is administered at a later time. Under the experimental condition, the tissue levels of mescaline were identical to those observed when CPZ was administered before two doses of mescaline. The interaction between an antipsychotic agent and a hallucinogen may have a potential significance from a pharmacological viewpoint.

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