Effects of d-, l- and
dl-Chlorpheniramine on Dopamine and
3, 4-Dihydroxyphenylacetic Acid Levels
in the Regional Parts of Rat Brain

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The purpose of this study was to further investigate the differences in the
effects of optical isomers of chlorpheniramine (d-, l- and dl-forms) on the levels of
dopamine (DA) and 3, 4-dihydroxyphenylacetic acid (DOPAC) in the regional
parts of rat brain. After the intravenous administration of each form of chlor-
pheniramine at a dose of 20 mg/kg, the DA and DOPAC levels were measured by
HPLC system. Each form of chlorpheniramine is effective in reducing DOPAC,
but not DA levels in the brain of rats. The degree of reducing DOPAC levels does
not correlate with the antihistaminic potency of these drugs. Thus, the present
results indicate that there is a lack of stereospecificity in reducing DOPAC levels,
suggesting the lack of stereospecificity in inhibiting DA reuptake. ——— chlor-
pheniramine; dopamine; 3, 4-dihydroxyphenylacetic acid; histamine H₁-blocker

Chlorpheniramine is known as a widely used potent histamine H₁-receptor
blocker and has optical isomers (Nauta and Rekker 1978). Previously, we reported
the differences in the transfer to rat brain of d-, l- and dl-chlorpheniramine
(Onodera et al. 1987; Sakurai et al. 1990, 1991). Clinical reports showed that
histamine H₁-receptor blockers are useful in treatment of Parkinson’s disease and
akathisia (Giar and Ducey 1950; Sato et al. 1989). Since antihistamines have
several side actions on both central and peripheral nervous systems which effects
are not related to inhibit the histamine H₁-receptors (Symchowicz et al. 1971;
Tuomisto and Tuomisto 1980), it is not clear whether only the histaminergic
system and/or more than two components might contribute to the clinical efficacy
of Parkinson’s disease. For example, Symchowicz et al. (1971) reported that
clorpheniramine and its derivatives block dopamine (DA) uptake into
synaptosomes of rat corpus striatum to various degrees in vitro. Moreover,
Shishido et al. (1991) have reported in vivo that chlorpheniramine significantly decreased levels of 3, 4-dihydroxyphenylacetic acid (DOPAC), although it had no effect on the levels of DA in whole mouse brain. Thus, it is suggested that this compound may have a possible use as an antiparkinson's agent besides the antihistamines. In this study, we tried to investigate the differences in the effects of optical isomers of chlorpheniramine on the levels of DA and DOPAC in the regional parts of rat brain.

**Materials and Methods**

**Animal**

Male Wistar rats, 130-150 g were purchased from Japan SLC, Inc. The animals were housed at a constant temperature (23±1°C) and a constant relative humidity (55±5%), and the light cycle was automatically controlled (7:00-19:00). The animals were given water and diet ad lib before the start of experiment. Twenty four hours before the neurochemical study, the rats were fasted but provided water ad lib.

**Drugs**

$d$-Chlorpheniramine maleate (Kowa, Nagoya), $d$-chlorpheniramine maleate (Yoshitomi Pharmaceutical Industry, Osaka) and $l$-chlorpheniramine maleate (Schering, Bloomfield, NJ, USA) were kindly donated from their companies. These drugs were dissolved in 0.9% saline solution and injected intravenously (i.v.) at a dose of 20 mg/kg in a volume of 0.1 ml/100 g body weight. The doses are expressed as weight of salts.

**The measurements of dopamine and 3, 4-dihydroxyphenylacetic acid by HPLC**

The rats were slightly anesthetized by ether and were injected saline or chlorpheniramine intravenously. The rats were decapitated at defined intervals after the injections, and their brains were quickly removed and dissected on ice into regional parts by the method of Glowinski and Iversen (1966) with a slight modification (Onodera et al. 1988), frozen on dry ice, and stored in a deep freezer until assay (−80°C). After homogenization of brain samples in 0.4 N perchloric acid containing 2 mM EDTA by a sonicator (Sonifier 450; BRANSON, Danbury, CT, USA) for 10 sec in an ice bath, the homogenate was centrifuged at 10,000 x g for 20 min at 4°C, and then stored at −80°C until use. The clear supernatant (20 µl) was applied to a high performance liquid chromatographic (HPLC) system with an electrochemical detector for measurements of DA and DOPAC levels, as described by Murai et al. (1988). The HPLC system consisted of a pump (CCPD; Tosoh, Tokyo), ODS-A (250×4.6 mm inside diameter; Yanagimoto, Kyoto), and electrochemical detector (EC-8000; Tosoh, Tokyo).

**Statistics**

Statistical significance was evaluated by Student's t-test.

**Results**

The effects of $d$-, $l$- and $dl$-chlorpheniramine on DA levels in regional parts of rat brain were firstly examined. The rats were administered i.v. with 20 mg/kg body weight of either $d$-, $l$- and $dl$-forms of chlorpheniramine and killed 0, 2, 5, 10, 30, 60 and 120 min later. The levels of DA in all regions measured were not affected by the i.v. administration of each form of chlorpheniramine (data not shown).
Figs. 1–3 show the effects of d-, l- and dl-chlorpheniramine on DOPAC levels in regional parts of rat brain. In the hypothalamus, each form of chlorpheniramine produced a drastic reduction (about 70 to 80%) in the DOPAC levels at each period when compared with the saline-treated group (Fig. 1). The levels of DOPAC in the striatum and brainstem were decreased by about 30 and 60% at 60 to 120 min after the administration of each form of chlorpheniramine, respectively.

Fig. 1. Effect of d-, l- and dl-chlorpheniramine maleate on 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the hypothalamus of rats. Each column and bar represents the mean ± s.e. (n = 6–12). Abscissa: hours after the i.v. injections of each form of chlorpheniramine. Ordinate: 3,4-dihydroxyphenylacetic acid (DOPAC) levels (ng/g). Rats were injected i.v. with saline or each form of chlorpheniramine maleate (20 mg/kg). (□) saline; (■) d-form; (●) l-form; (○) dl-form. Statistical difference between saline and each form of chlorpheniramine was assessed by Student's t-test (*p < 0.05, **p < 0.01).

Fig. 2. Effect of d-, l- and dl-chlorpheniramine maleate on 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the striatum of rats. See the legend to Fig. 1. Statistical difference between saline and each form of chlorpheniramine was assessed by Student's t-test (*p < 0.05, **p < 0.01).
There were no significant differences in reducing DOPAC levels between d-, l- and dl-forms of chlorpheniramine in these regions (Figs. 1-3). In the cortex, hippocampus and amygdala, the DOPAC levels were not affected by each form of chlorpheniramine (data not shown).

DISCUSSION

Watanabe et al. (1984) showed that histaminergic neurons are detected immunohistochemically in the brain of rats. There is a fairly widespread distribution of nerve fibers emanating from cell bodies in the hypothalamus and histaminergic fibers are especially dense in the limbic system (Watanabe et al. 1985). Quach et al. (1980) and Palacios et al. (1981) reported the similar distribution of H1-receptors studied with [3H]-mepyramine as a ligand. Previous our pharmacokinetic studies indicated that the highest distribution of dl-form of chlorpheniramine was observed in the limbic system (Onodera 1987; Onodera et al. 1987), and indicated that there is a lack of stereospecificity in the distribution of chlorpheniramine in the rat brain (Onodera et al. 1987; Sakurai et al. 1990, 1991).

Under these circumstances, each form of chlorpheniramine is effective in reducing DOPAC, but not DA levels in the brain of rats. This result is consistent with that of mice after the intraperitoneal injection of the d-form (Shishido et al. 1991). In the present experiment, it is notable that the degree of reducing DOPAC levels does not correlate with the antihistaminic potency of each form of chlorpheniramine. d-Chlorpheniramine has been shown to be some 200 times more effective than its enantiomer in vivo in protecting guinea-pig against histamine (Roth and Govier 1958). In the studies of inhibition of [3H]mepyra-
Chlorpheniramine and DOPAC

Antihistamines such as cyproheptadine, diphenhydramine and promethazine are known to affect other neuron systems such as dopamine, noradrenaline, serotonin and so on (Stone et al. 1961; Brown and Vernikos 1980; Tuomisto and Tuomisto 1980; Shishido et al. 1991). Chlorpheniramine also influence the reuptake of DA (Symchowicz et al. 1971), serotonin and noradrenaline (Lidbrink et al. 1971; Shishido et al. 1991). The present experiments showed that the each form of chlorpheniramine reduced DOPAC levels in vivo. Moreover, chlorpheniramine potentiated the action of amphetamine in self-stimulation in rats (Symchowicz et al. 1971) or apomorphine-induced gnawing in mice (Dadkar et al. 1976). These data are completely in line with the idea that chlorpheniramine has the capacity of dopamine reuptake inhibition into synaptosomes of the brain. In addition, each form of chlorpheniramine reduced DOPAC levels almost equally in the hypothalamus, striatum and brainstem in our results. Thus, the present results indicate that there is a lack of stereospecificity in chlorpheniramine in reducing DOPAC levels, suggesting the lack of stereospecificity in inhibiting DA uptake. Although these findings raise the problems about the possible physiological and behavioral effects by using chlorpheniramine in animal experiments, antihistamines which have such kind of side effects will not be regarded as useless to treat the depressed patients or Parkinson's disease. Therefore, further investigation will be expected in the clinical efficacy of this type of antihistamines.

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References

discrete brain areas of mice within ten minutes by HPLC with electrochemical detection. J. Neurochem., 50, 473–479.