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

EIICHI SAKURAI, SEIJI YAMASAKI, NOBORU HIKICHI and KENJI ONODERA*

Department of Pharmaceutics I, Tohoku College of Pharmacy, Sendai 981, and *Department of Pharmacology, Tohoku University School of Dentistry, Sendai 980

SAKURAI, E., YAMASAKI, S., HIKICHI, N. and ONODERA, K. *Effects of d-, l- and dl-Chlorpheniramine on Dopamine and 3, 4-Dihydroxyphenylacetic Acid Levels in the Regional Parts of Rat Brain.* Tohoku J. Exp. Med., 1991, 163 (4), 239-244
— 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 chlorpheniramine 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. ——— chlorpheniramine ; 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 chlorpheniramine 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 ($23 \pm 1^\circ\text{C}$) and a constant relative humidity ($55 \pm 5\%$), 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

dl-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 $10,000 \times g$ 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 $\times$ 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 $\pm$ 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). (stippled) saline; (white) *d*-form; (dotted) *l*-form; (hatched) *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 (* $p < 0.05$, ** $p < 0.01$).

Fig. 3. Effect of *d*-, *l*- and *dl*-chlorpheniramine maleate on 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the brainstem of rats. See the legend to Fig. 1. Statistical difference between saline and each form of chlorpheniramine was assessed by Student's *t*-test (* $p < 0.05$).

(Figs. 2 and 3). 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 H_1 -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 intraperitoneally 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]mepyr-

amine binding in vivo and vitro, *d*-chlorpheniramine showed more 100-fold differences with that of the *l*-form (Hill et al. 1978).

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.

Acknowledgments

We would like to thank Prof. T. Watanabe (Department of Pharmacology I, Tohoku University School of Medicine) for his critical comments.

References

- 1) Brown, P.A. & Vernikos, J. (1980) Antihistamine effect on synaptosomal up take of serotonin, norepinephrine and dopamine. *Eur. J. Pharmacol.*, **65**, 89-92.
- 2) Dadkar, N.K., Dohadwalla, A.N. & Bhattacharya, B.K. (1976) Influence of pheniramine and chlorpheniramine on apomorphine induced gnawing in mice. *Psychopharmacology*, **48**, 7-10.
- 3) Giar, D.C. & Ducey, J. (1950) Chemical structure of substances effective in treatment of parkinsonism. *Arch. Intern. Med.*, **85**, 284-298.
- 4) Glowinski, J. & Iversen, L.L. (1966) Regional studies of catecholamines in the rat brain. I. The disposition of [³H]norepinephrine, [³H]dopamine and [³H]dopa in various regions of the brain. *J. Neurochem.*, **13**, 655-669.
- 5) Hill, S.J., Emson, P.C. & Young, J.M. (1978) The binding of [³H]mepyramine to histamine H1 receptors in guinea-pig brain. *J. Neurochem.*, **31**, 997-1004.
- 6) Lidbrink, P., Jonsson, G. & Fuxe, K. (1971) The effect of imipramine like drugs and antihistamine drug on uptake mechanisms in the central noradrenaline and 5-hydroxytryptamine neurons. *Neuropharmacology*, **10**, 521-536.
- 7) Murai, S., Saito, H., Masuda, Y. & Itoh, T. (1988) Rapid determination of norepinephrine, dopamine, serotonin, their precursor amino acids, and related metabolites in

- discrete brain areas of mice within ten minutes by HPLC with electrochemical detection. *J. Neurochem.*, **50**, 473-479.
- 8) Nauta, W.T. & Rekker, R.F. (1978) Structure-activity relationship of H₁-receptor antagonists. In: *Handbook of Experimental Pharmacology: Histamine II and Antihistaminics* edited by M. Rocha e Silva, Springer-Verlag, Berlin, pp. 234-239.
  - 9) Onodera, K. (1987) Muricidal suppression by chlorpheniramine and changes in brain levels following dietary-induced thiamine deficiency in rats. *Physiol. Behav.*, **41**, 71-78.
  - 10) Onodera, K., Sakurai, E. & Niwa, H. (1987) Effect of chlorpheniramine on muricide induced by thiamine deficiency: Pharmacokinetic and behavioral studies. *Agents Actions*, **20**, 229-232.
  - 11) Onodera, K., Maeyama, K. & Watanabe, T. (1988) Regional changes in brain histamine levels following dietary-induced thiamine deficiency in rats. *Jpn. J. Pharmacol.*, **47**, 323-326.
  - 12) Palacios, J.M., Wamsley, J.K. & Kuhar, M.J. (1981) The distribution of histamine H₁-receptors in the rat brain: Autoradiographic study. *Neuroscience*, **6**, 15-37.
  - 13) Quach, T.T., Duchemin, A.M., Rose, C. & Schwartz, J.C. (1980) Labeling of histamine H₁-receptors in the brain of the living mouse. *Neurosci. Lett.*, **17**, 49-54.
  - 14) Roth, F.E. & Govier, W.M. (1958) Comparative pharmacology of chlorpheniramine (chlor-trimeton) and its optical isomers. *J. Pharmacol. Exp. Ther.*, **124**, 347-349.
  - 15) Sakurai, E., Hikichi, N., Niwa, H., Yamasaki, S. & Maeyama, K. (1990) Differences in the transfer to rat brain of *d*- and *l*-chlorpheniramine and regional changes in brain histamine concentrations. *Eur. J. Pharmacol.*, **183**, 712.
  - 16) Sakurai, E., Yamasaki, S., Izuka, Y., Hikichi, N. & Niwa, H. (1991) The disposition of enantiomers. V. A method for the optical resolution of chlorpheniramine and its stereoselective pharmacokinetics. *J. Pharmacobiodyn.*, **14**, in press.
  - 17) Sato, M., Saito, H., Matsumoto, H., Matsuoka, H., Matsumoto, J., Ueno, T., Fuse, Y., Matue, Y., Kodama, M. & Maeyama, K. (1989) Correlation between plasma histamine levels and improvement of neuroleptic-induced akathisia. *Annu. Rep. Pharmacopsychi. Res. foun.*, **21**, 302-308.
  - 18) Shishido, S., Oishi, R. & Saeki, K. (1991) In vivo effects of some histamine H₁-receptor antagonists on monoamine metabolism in the mouse brain. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **343**, 185-189.
  - 19) Stone, C.A., Wenger, H.C., Ludden, C.T., Stavorski, J.M. & Ross, C.A. (1961) Antiserotonin-antihistaminic properties of cyproheptadine. *J. Pharmacol. Exp. Ther.*, **13**, 73-84.
  - 20) Symchowicz, S., Korduba, C.A. & Veals, J. (1971) Inhibition of dopamine uptake into synaptosomes of rat corpus striatum by chlorpheniramine and its structural analogues. *Life Sci.*, **10**, 35-42.
  - 21) Tuomisto, J. & Tuomisto, L. (1980) Effects of histamine and histamine antagonists on the uptake and release of catecholamines and 5-HT in brain synaptosomes. *Med. Biol.*, **58**, 33-37.
  - 22) Watanabe, T., Taguchi, Y., Shiosaka, S., Tanaka, J., Kubota, H., Terano, Y., Tohyama, M. & Wada, H. (1984) Distribution of the histaminergic neuron system of rats; a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. *Brain Res.*, **295**, 13-25.
  - 23) Watanabe, T., Taguchi, Y., Takeda, N., Shiosaka, S., Tohyama, M. & Wada, H. (1985) Purification and antibodies against L-histidine decarboxylase. In: *Advances in Biosciences*, Vol. 51, *Frontiers in Histamine Research*, edited by C.R. Ganellin & J.C. Schwartz, Pergamon Press, Oxford, pp. 225-235.
-