British Journal of Pharmacology (2002) 136, 193–200

www.nature.com/bjp

Carvedilol attenuates neuroleptic-induced orofacial dyskinesia: possible antioxidant mechanisms

[1]Pattipati S. Naidu, [1]Amanpreet Singh & *,[1]Shrinivas K. Kulkarni

[1]Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160014, India

1 Tardive dyskinesia (TD), a syndrome of potentially irreversible, involuntary hyperkinetic disorder occurring in 20–40% of the patient population undergoing chronic neuroleptic treatment is a major limitation of neuroleptic therapy.

2 Oxidative stress and products of lipid peroxidation are implicated in the pathophysiology of various neurological disorders including tardive dyskinesia.

3 Chronic treatment with neuroleptics leads to the development of abnormal oral movements in rats known as vacuous chewing movements (VCMs). Vacuous chewing movements in rats are widely accepted as an animal model of tardive dyskinesia.

4 All the antipsychotics were administered i.p. once daily for 21 days, whereas carvedilol (also i.p.) was administered twice daily. Rats chronically treated with haloperidol (1.0 mg kg^{-1}) or chlorpromazine (5 mg kg^{-1}) but not clozapine (2 mg kg^{-1}) significantly developed vacuous chewing movements and tongue protrusions. Carvedilol dose dependently (0.5–2 mg kg^{-1}) reduced the haloperidol or chlorpromazine-induced vacuous chewing movements and tongue protrusions.

5 Biochemical analysis revealed that chronic haloperidol or chlorpromazine but not clozapine treatment significantly induced lipid peroxidation and decreased the glutathione (GSH) levels in the forebrains of rats. Chronic haloperidol or chlorpromazine but not clozapine treated rats showed decreased forebrain levels of antioxidant defence enzymes, superoxide dismutase (SOD) and catalase.

6 Co-administration of carvedilol (0.5–2 mg kg^{-1}) significantly reduced the lipid peroxidation and restored the decreased glutathione levels by chronic haloperidol or chlorpromazine treatment. Co-administration of carvedilol (1–2 mg kg^{-1}) significantly reversed the haloperidol or chlorpromazine-induced decrease in forebrain SOD and catalase levels in rats. However, lower dose of carvedilol (0.5 mg kg^{-1}) failed to reverse chronic haloperidol or chlorpromazine-induced decrease in forebrain SOD and catalase levels.

7 The major findings of the present study suggest that oxidative stress might play a significant role in neuroleptic-induced orofacial dyskinesia. In conclusion, carvedilol could be a useful drug for the treatment of neuroleptic-induced orofacial dyskinesia.

British Journal of Pharmacology (2002) **136,** 193–200

Keywords: Oxidative stress; tardive dyskinesia; VCMs; neuroleptics; carvedilol

Abbreviations: GSH, glutathione; SOD, superoxide dismutase; TD, tardive dyskinesia; VCMs, vacuous chewing movements

Introduction

Neuroleptics are used extensively in the treatment of schizophrenia and other affective disorders. Unfortunately typical antipsychotics such as haloperidol and chlorpromazine often cause distressing side effects involving extrapyramidal tract. These adverse reactions comprise of a variety of movement disorders (Grohman *et al*., 1990) including tardive dyskinesia, which occurs in 20–40% of the patient population (Casey, 2000; Kulkarni & Naidu, 2001). Tardive dyskinesia, a syndrome of potentially irreversible, involuntary hyperkinetic disorders that occurs during chronic neuroleptic treatment, is a major limitation of neuroleptic therapy (Egan *et al*., 1997; Casey, 2000). In spite of its high frequency of occurrence, the exact mechanism underlying the pathophysiology of tardive dyskinesia is not known. It has been hypothesized that dopamine receptor supersensitivity arising from upregulation of dopamine D_2 receptors following neuroleptic therapy could be the reason for the development of tardive dyskinesia. However, support for this hypothesis is dissipating (Fibiger & Lloyd, 1984; Andreassen & Jorgensen, 2000). Several other alternative hypotheses have been proposed and different suppressive agents have been used but with limited success (Egan *et al*., 1997; Gupta *et al*., 1999).

Oxidative stress and products of lipid peroxidation are implicated in the pathophysiology of various neurological disorders. Chronic treatment with neuroleptics increases free radical production and oxidative stress (Balijepalli *et al*., 2001). A role for increased reactive oxygen species and oxidative stress in the etiopathology of neuroleptic-induced tardive dyskinesia has been proposed (Cadet *et al*., 1986; Coyle & Puttfarcken, 1993). Administration of single dose of haloperidol to mice led to increase oxidized glutathione (GSSG) levels in the striatum indicating generation of oxidative stress by the drug (Cohen & Spina, 1988). Elkashef & Wyatt (1999) have reported that rats with vacuous chewing movement had significantly higher thiobarbituric acid

*Author for correspondence; E-mail: skpu@yahoo.com

reactive substances (TBARS) in the striatum, suggesting increased lipid peroxidation and free radical production in these animals. Chronic use of neuroleptics is also reported to cause decrease in the activity of antioxidant defence enzymes superoxide dismutase (SOD) and catalase (Cadet *et al*., 1987). Free radicals are through to play a role in ageing process, and age is one of the risk factors for the development of TD. Richardson *et al*. (1986) reported that there was a positive correlation between increasing age and the development of TD, further supporting the role of free radicals in the pathogenesis of TD. Vitamin E, an antioxidant and free radical scavenger has been reported to be effective in the treatment of TD (Gupta *et al*., 1999; Soares & McGrath, 2000; Elkashef & Wyatt, 1999). All these accumulating data strongly support the free radical hypothesis of TD.

Carvedilol, 1-[carbazole(4)-oxy]3-[2-methoxy-phenoxyethyl)-amino]-2-propanol, is a lipophilic nonselective β-adrenoceptor antagonist with strong antioxidant effect (Yue *et al*., 1992a; Abreu *et al*., 2000; Noguchi *et al*., 2000; Yoshioka *et al*., 2000). Carvedilol and certain of its metabolites are potent antioxidants. The antioxidant activity of carvedilol has been attributed to the carbazole moiety of the drug (Feuerstein *et al*., 1997). The antioxidant effects of carvedilol have been demonstrated and characterized in a variety of *in vitro* test systems, and have also been confirmed *in vivo* in animals and in humans (Ma *et al*., 1996; Rabasseda, 1998). Carvedilol has been shown to scavenge free radicals and inhibit lipid peroxidation in swine ventricular membranes (Yue *et al*., 1992b), rat brain homogenates (Yue *et al*., 1992a), human low-density lipoproteins (Yue *et al*., 1995). The capacity of carvedilol to scavenge oxygen free radicals in both lipid and aqueous environments has been confirmed using electron spine traps to generate EPR spectra. Carvedilol has been reported to prevent Fe^{2+}/vitamin C-induced depletion of α-tocopherol from brain homogenates (Yue *et al*., 1992a; Lysko *et al*., 2000). Carvedilol is approximately 10 fold more potent as an antioxidant than vitamin E. Several metabolites of carvedilol most notably SB-209995, are extremely potent antioxidants, being 30 to 80 fold more potent than carvedilol and up to 1000 fold more potent than vitamin E (Feuerstein & Yue, 1994; Feuerstein *et al*., 1997).

In the present study we have investigated the effect carvedilol on neuroleptic-induced orofacial dyskinesia in rats, a potential animal model for tardive dyskinesia.

Methods

Animals

Male Wistar rats, bred in the Central Animal House facility of the Panjab University and weighing between 180–220 g, were used. The animals were housed under standard laboratory conditions, maintained on a 12-h light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the test. Each animal was used only once in the experiments. All experiments were carried out between 0900 and 1500 h. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Induction of orofacial dyskinesia

Haloperidol (1.0 mg kg^{-1} i.p.), chlorpromazine (5.0 mg kg^{-1} i.p.) or clozapine (2.0 mg kg^{-1} i.p.) was given chronically to rats for a period of 21 days to induce oral dyskinesia (Sasaki *et al*., 1995; Naidu & Kulkarni, 2001a, b). All the behavioural assessments were carried out after 24 h of the last dose of haloperidol.

Behavioural assessment of orofacial dyskinesia

On the test day rats were placed individually in a small ($30 \times 20 \times 30$ cm) Plexiglas cage for the assessment of oral dyskinesia. Animals were allowed 10 min to get used to the observation cage before behavioural assessments. To quantify the occurrence of oral dyskinesia, hand operated counters were employed to score tongue protrusion and vacuous chewing frequencies. In the present study vacuous chewing movements are referred to as single mouth openings in the vertical plane not directed toward physical material. If tongue protrusion, vacuous chewing movements occurred during a period of grooming, they were not taken into account. Counting was stopped whenever the rat began grooming, and restarted when grooming stopped. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of oral dyskinesia when the animal was faced away from the observer. The behavioural parameters of oral dyskinesia were measured continuously for a period of 5 min. In all the experiments the scorer was unaware of the treatment given to the animals.

Dissection and homogenization

On the 22nd day of haloperidol treatment, the animals were sacrificed by decapitation immediately after behavioural assessments. The brains were removed, forebrain was dissected out and rinsed with isotonic saline and weighed. A 10% (w v^{-1}) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The post nuclear fraction for catalase assay was obtained by centrifugation of the homogenate at $1000 \times g$ for 20 min, at 4°C and for other enzyme assays centrifuged at $12{,}000 \times g$ for 60 min at 4°C.

Lipid peroxidation assay

The quantitative measurement of lipid peroxidation in forebrain was performed according to the method of Wills (1966). The amount of malondialdehyde (MDA) formed was measured by the reaction with thiobarbituric acid at 532 nm using Perkin Elmer lambda 20 spectrophotometer. The results were expressed as nmol of malondialdehyde/mg protein using the molar extinction coefficient of chromophore (1.56×10^5 M^{-1} cm^{-1}).

Estimation of reduced glutathione

Reduced glutathione in the forebrain was estimated according to the method of Ellman (1959). A 0.75 ml of homogenate was precipitated with 0.75 ml of 4% sulphosalicylic acid. The samples were centrifuged at $1200 \times g$ for 15 min at 4°C. The assay mixture contained 0.5 ml of supernatant and 4.5 ml of 0.01 M (in 0.1 M phosphate buffer, pH 8.0) DTNB (5-5′-DithioBis-(2-Nitrobenzoic acid)). The

yellow colour developed was read immediately at 412 nm using Perkin Elmer lambda 20 spectrophotometer. The results were expressed as nmol of GSH per mg protein.

Enzyme assays

Superoxide dismutase activity Superoxide dismutase activity was assayed according to the method of Kono (1978), wherein the reduction of nitazoblue tetrazolium (NBT) was inhibited by the superoxide dismutase is measured at 560 nm using Perkin Elmer lambda 20 spectrophotometer. Briefly the reaction was initiated by the addition of hydroxylamine hydrochloride to the reaction mixture containing NBT and post nuclear fraction of fore brain homogenate. The results were expressed as units/mg protein, where one unit of enzyme is defined as the amount of enzyme inhibiting the rate of reaction by 50%.

Catalase activity Catalase activity was assayed by the method of Luck (1971), wherein the breakdown of H_2O_2 being measured at 240 nm. Briefly, the assay mixture consisted of 3 ml of H_2O_2 phosphate buffer (1.25×10^{-2} H_2O_2 m) and 0.05 ml of supernatant of forebrain homogenate (10%) and the changes in absorbance were recorded at 240 nm using Perkin Elmer lambda 20 spectrophotometer. Enzyme activity was calculated using the millimolar extinction coefficient of H_2O_2 (0.07). The results were expressed as μmol H_2O_2 decomposed/min/mg protein.

Protein estimation The protein content was measured according to the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Drugs and treatment schedule

The following drugs were used in the present study. Haloperidol (Serenace® Inj., Searle India, India) was diluted with distilled water. Chlorpromazine (May & Baker, India) and clozapine (Torrent Pharma, India) were dissolved in distilled water. Carvedilol (Zydus Medica, India) was suspended in distilled water wetted with Tween 80. All drugs were administered intraperitoneally in a constant volume of 0.5 ml per 100 g of body weight of rat. Animals were divided into four groups. First group received vehicle, second group received haloperidol, chlorpromazine or clozapine plus vehicle for carvedilol, third group received haloperidol-, chlorpromazine- or clozapine plus carvedilol and the fourth group received carvedilol only. Haloperidol, chlorpromazine or clozapine was administered once daily in the morning (0900 h) and this group also received vehicle for carvedilol twice daily. Carvedilol was given twice daily (0900 and 1700 h) for a period of 21 days and behavioural assessments were measured 24 h after the last dose. Drug doses were selected on the basis of previous studies conducted in our laboratory and those reported in the literature.

Statistical analysis

One specific group of rats was assigned to one specific drug treatment condition and each group comprised six rats ($n=6$). All the values are expressed as means$\pm$s.e.mean. The data were analysed by using analysis of variance (ANOVA) followed by Dunnett's test. In all tests, the criterion for statistical significance was $P<0.05$.

Results

Behavioural effects of chronic haloperidol, chlorpromazine, clozapine or carvedilol treatment in rats

Chronic haloperidol or chlorpromazine but not clozapine treatment significantly increased the vacuous chewing movement and tongue protrusions frequency in rats as compared to vehicle treated controls. Carvedilol alone did not induce any VCMs or tongue protrusions (Table 1).

Biochemical effects of chronic haloperidol, chlorpromazine, clozapine or carvedilol treatment in rats

Chronic haloperidol or chlorpromazine but not clozapine treated animals showed decreased levels of glutathione and increased levels of lipid peroxidation products as compared to vehicle treated control animals. Chronic haloperidol or chlorpromazine but not clozapine treated animals showed low levels of detoxifying enzymes such as SOD and catalase as compared to vehicle treated control animals. Carvedilol alone did not induce any biochemical alterations (Table 2).

Effect of carvedilol on haloperidol or chlorpromazine-induced vacuous chewing movements and tongue protrusions

Chronic treatment with carvedilol dose dependently (0.5–2 mg kg^{-1}) suppressed the haloperidol (Figure 1A,B) or chlorpromazine-induced (Figure 2A,B) vacuous chewing movements and tongue protrusions.

Effect of carvedilol on the forebrain MDA level in chronic haloperidol or chlorpromazine treated rats

Chronic haloperidol or chlorpromazine but not clozapine treatment for 21 days induced lipid peroxidation as indicated by a significant raise in forebrain MDA levels as compared to vehicle treated rats (Table 2). Co-administration of carvedilol (0.5–2 mg kg^{-1}) along with haloperidol (Table 3) or chlorpromazine (Table 4) significantly reversed the extent of

Table 1 Behavioural effects of chronic haloperidol, chlorpromazine, clozapine or carvedilol treatment in rats

Treatment (mg kg^{-1})	*VCMs/5 min*	*Tongue protrusions/5 min*
Vehicle	6$\pm$1	2$\pm$0.577
Haloperidol (1)	58.667$\pm$3.667*	22.833$\pm$1.167*
Chlorpromazine (5)	52.833$\pm$2.667*	19.667$\pm$2.167*
Clozapine (2)	8.233$\pm$2.132	3$\pm$1.22
Carvedilol (0.5)	6$\pm$0.899	3.133$\pm$0.931
Carvedilol (1)	7.133$\pm$1.133	3$\pm$0.577
Carvedilol (2)	6.233$\pm$1.33	2.266$\pm$0.663

Values expressed as mean$\pm$s.e.mean of six animals. *$P<0.05$ as compared to vehicle treated group (ANOVA followed by Dunnett's test).

Table 2 Biochemical effects of chronic haloperidol, chlorpromazine, clozapine or carvedilol treatment in rats

Treatment	*Lipid peroxidation* (nmol MDA/mgpr)	*GSH* *nmol mgpr^{-1}* (% of control)	*SOD* *units mgpr^{-1}* (% of control)	*Catalase* *μmol H_2O_2 decomposed min mgpr^{-1}* (% of control)
Vehicle	1.343±0.081	100±2.53	100±2.6	100±9.23
Haloperidol (1)	3.498±0.132*	47.24±3.32*	32.43±2.45*	44.25±4.94*
Chlorpromazine (5)	3.028±0.082*	52.37±4.32*	38.23±3.45*	49.23±3.94*
Clozapine (2)	1.597±0.079	97.22±4.32	98.4±4.63	94.6±6.323
Carvedilol (0.5)	1.296±0.032	98.34±3.36	99.32±3.25	96.69±7.12
Carvedilol (1)	1.432±0.132	97.39±4.133	97.26±3.96	102.03±5.32
Carvedilol (2)	1.396±0.129	101.25±2.233	99.63±3.163	110.7±4.33

Values expressed as mean±s.e.mean of six animals in case of lipid peroxidation and values expressed as per cent response of vehicle treated control group in case of GSH, SOD and catalase. *$P<0.05$ as compared to vehicle treated group (ANOVA followed by Dunnett's test).

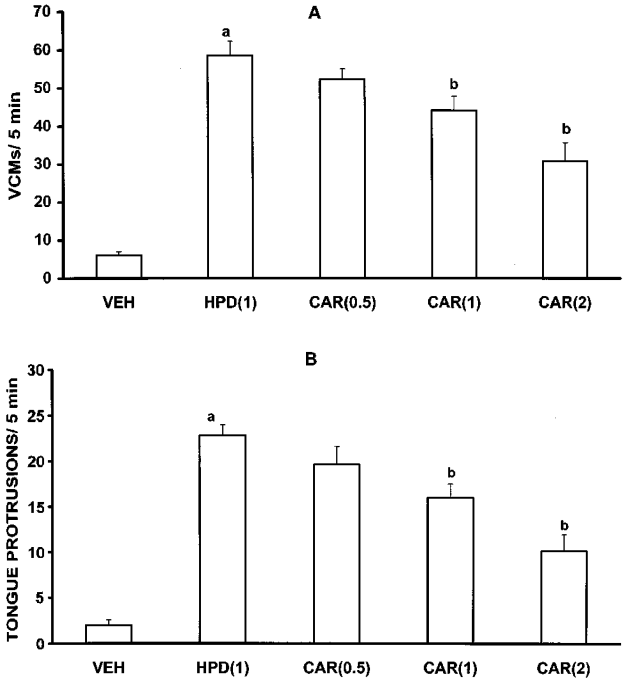

Figure 1 Effect of co-administration of carvedilol (CAR) on chronic haloperidol (HPD)-induced vacuous chewing movements (A) and tongue protrusions (B) in rats. Values expressed as means±s.e.m. [a]$P<0.05$ as compared to vehicle treated group. [b]$P<0.05$ as compared to haloperidol treated group (ANOVA followed by Dunnett's test).

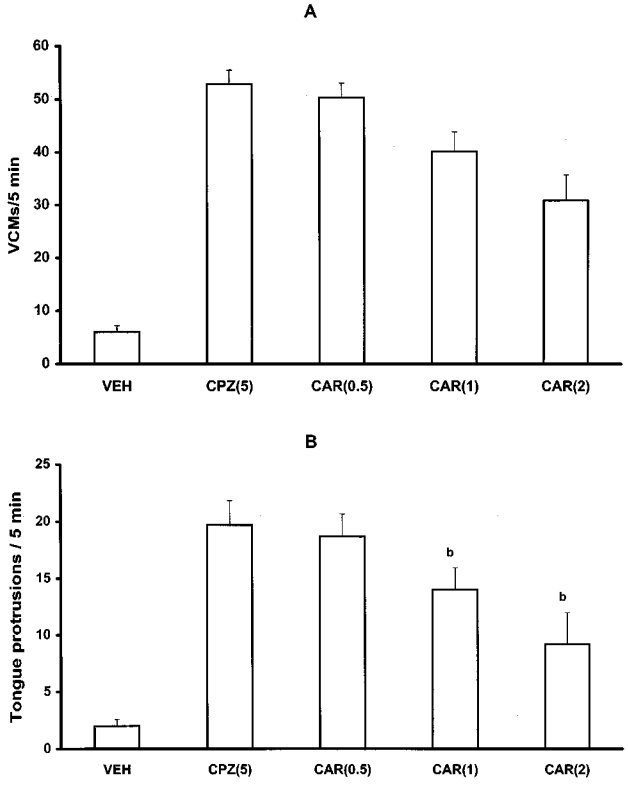

Figure 2 Effect of co-administration of carvedilol (CAR) on chronic chlorpromazine (CPZ)-induced vacuous chewing movements (A) and tongue protrusions (B) in rats. Values expressed as mean±s.e.m. [a]$P<0.05$ as compared to vehicle treated group. [b]$P<0.05$ as compared to chlorpromazine treated group (ANOVA followed by Dunnett's test).

lipid peroxidation as compared to haloperidol only or chlorpromazine only treated rats.

Effect of carvedilol on the forebrain glutathione (GSH) levels in chronic haloperidol or chlorpromazine treated rats

Chronic administration of haloperidol or chlorpromazine but not clozapine significantly decreased the forebrain GSH levels (Table 2). Co-administration of carvedilol (1–2 mg kg^{-1} twice daily for 21 days) significantly reversed the haloperidol (Figure 3A) or chlorpromazine-induced (Figure 3B) decrease in the forebrain GSH levels. However, a lower dose of carvedilol (0.5 mg kg^{-1}) failed to reverse chronic haloperidol or chlorpromazine-induced decrease in forebrain GSH levels.

Effect of carvedilol on the forebrain antioxidant enzyme levels in chronic haloperidol or chlorpromazine treated rats

Chronic haloperidol of chlorpromazine but not clozapine treated rats showed decreased levels of antioxidant enzymes SOD and catalase in their forebrain homogenates (Table 2). Co-administration of carvedilol (1–2 mg kg^{-1} significantly reversed the haloperidol or chlorpromazine-induced decrease in forebrain SOD (Figure 4A,B) and catalase (Figure 5A,B) levels as compared to only haloperidol or chlorpromazine

Table 3 Effect of co-administration of carvedilol on chronic haloperidol-induced lipid peroxidation in the rat forebrain

Treatment (mg kg^{-1})	*Lipid peroxidation* (nmol malondialdehyde (MDA)/mg protein)
Vehicle	1.343±0.081
Haloperidol (1)	3.498±0.132*
Carvedilol (0.5)	2.820±0.126
Carvedilol (1)	2.258±0.095
Carvedilol (2)	2.012±0.024

Values expressed as mean±s.e.mean of six animals. *$P<0.05$ as compared to vehicle treated group. **$P<$ as compared to haloperidol treated group (ANOVA followed by Dunnett's test).

Table 4 Effect of co-administration of carvedilol on chronic chlorpromazine-induced lipid peroxidation in the rat forebrain

Treatment (mg kg^{-1})	*Lipid peroxidation* (nmol malondialdehyde (MDA)/mg protein)
Vehicle	1.343±0.081
Chlorpromazine (5)	3.028±0.082*
Carvedilol (0.5)	2.618±0.126**
Carvedilol (1)	2.018±0.095**
Carvedilol (2)	1.812±0.024**

Values expressed as mean±s.e.mean of six animals. *$P<0.05$ as compared to vehicle treated group. **$P<$ as compared to chlorpromazine treated group (ANOVA followed by Dunnett's test).

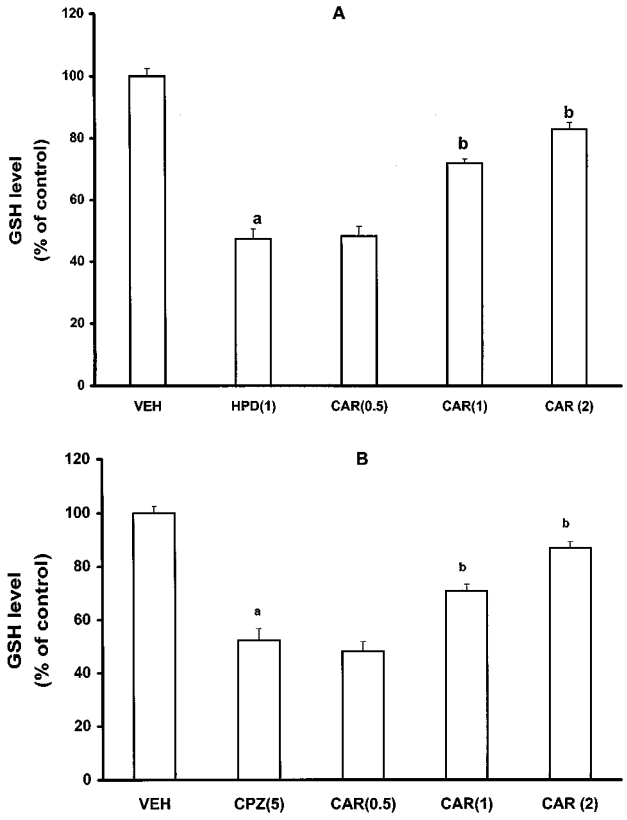

Figure 3 (A) Effect of co-administration of carvedilol (CAR) on chronic haloperidol (HPD)-induced glutathione (GSH) depletion in rats. Values expressed as per cent response of vehicle treated control group. Each value represents mean value of six animals. [a]$P<0.05$ as compared to vehicle treated control group. [b]$P<0.05$ as compared to haloperidol treated group (ANOVA followed by Dunnett's test). (B) Effect of co-administration of carvedilol (CAR) on chronic chlorpromazine (CPZ)-induced glutathione (GSH) depletion in rats. Values expressed as per cent response of vehicle treated control group. Each value represents mean value of six animals. [a]$P<0.05$ as compared to vehicle treated control group. [b]$P<0.05$ as compared to chlorpromazine treated group (ANOVA followed by Dunnett's test).

treated rats. However, a lower dose of carvedilol (0.5 mg kg^{-1}) failed to reverse chronic haloperidol or chlorpromazine-induced decrease in forebrain SOD and catalase levels.

Discussion

In the present study chronic haloperidol or chlorpromazine but not clozapine treated animals showed increased frequencies of vacuous chewing movements and tongue protrusions as compared to vehicle treated control animals. Chronic treatment with carvedilol significantly attenuated the induction of haloperidol, or chlorpromazine-induced vacuous chewing movements and tongue protrusion in a dose dependent fashion.

Existing evidence indicates that an unbalanced production of free radicals is associated with chronic neuroleptic use and might contribute to the onset of tardive dyskinesia and other movement disorders, such as dystonias and Parkinsonism (Cadet *et al*., 1986). This effect can be related, at least in part, to a reduction in specific endogenous antioxidant mechanisms, such as a decrease in GSH levels (Shivakumar & Ravindranath, 1993) and low levels of antioxidant defense enzymes such as SOD and catalase (Elkashef & Wyatt, 1999).

The molecular mechanisms by which neuroleptics increase oxygen free radical production are unknown. Neuroleptics act by blocking dopamine receptors (Creese *et al*., 1976). Such blockade could result in increased dopamine turnover, which in turn could conceivably lead to an increased production of hydrogen peroxide, resulting in oxidative stress (Cohen & Spina, 1988). Dopamine is primarily metabolized through oxidation by monoamine oxidase (MAO) to 3,4-dihydroxyphenyl-acetic acid (DOPAC). This reaction produces hydrogen peroxide. Dopamine is also metabolized by auto-oxidation yielding superoxide radical. Hydrogen peroxide can further react with iron or copper ions to produce the hydroxyl radical, which is the most toxic of free radicals. Increased dopamine turnover by neuroleptics could lead to excessive production of these potentially damaging free radicals (Elkashef & Wyatt, 1999). Oxygen free radicals are also reported to diminish the dopamine transporter function further increasing the extracellular dopamine levels (Fleckenstein *et al*., 1997). However, this does not seem to be the only mechanism responsible for the GSH/ATP depletion observed during haloperidol treatment (Vairetti *et al*., 1999).

Very recently, using rat primary cortical neurones and the mouse hippocampal cell line HT-22, Sagara (1998) showed that haloperidol causes a sequence of cellular alterations that leads to cell death, and that the production of reactive oxygen species (from mitochondria but not from the

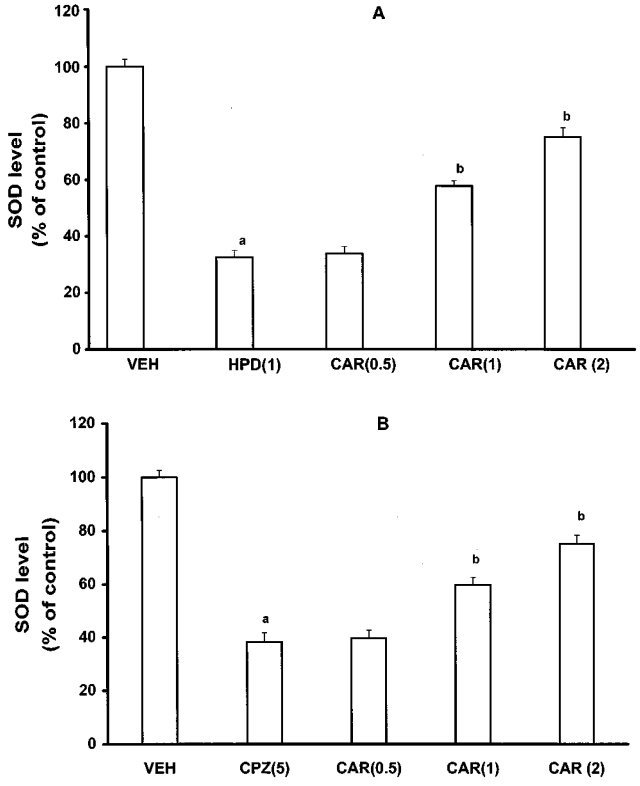

Figure 4 (A) Effect of co-administration of carvedilol (CAR) on chronic haloperidol (HPD) mediated depletion in the level of forebrain antioxidant enzyme superoxide dismutase (SOD). Values expressed as per cent response of vehicle treated control group. Each value represents mean value of six animals. $^{a}P<0.05$ as compared to vehicle treated control group. $^{b}P<0.05$ as compared to haloperidol treated group (ANOVA followed by Dunnett's test). (B) Effect of co-administration of carvedilol (CAR) on chronic chlorpromazine (CPZ) mediated depletion in the level of forebrain antioxidant enzyme superoxide dismutase (SOD). Values expressed as per cent response of vehicle treated control group. Each value represents mean value of six animals. $^{a}P<0.05$ as compared to vehicle treated control group. $^{b}P<0.05$ as compared to chlorpromazine treated group (ANOVA followed by Dunnett's test).

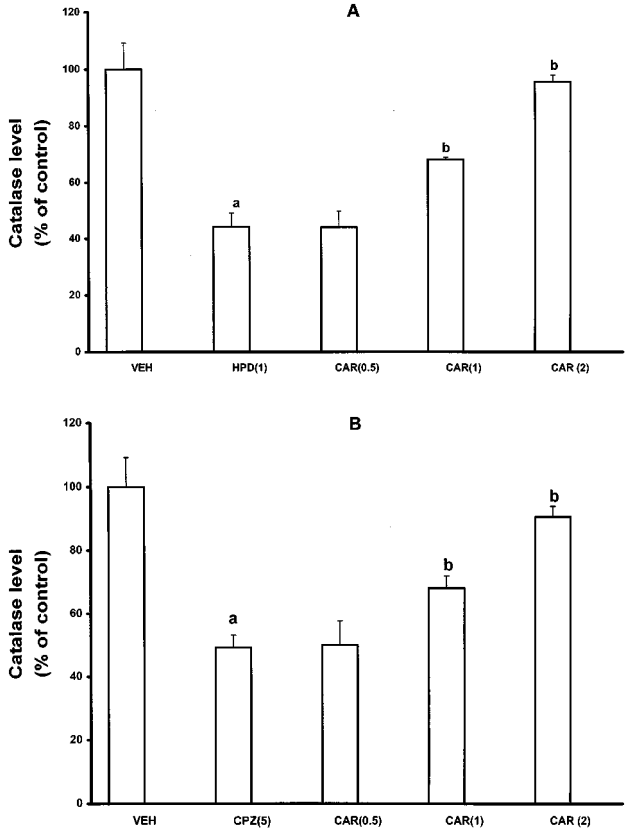

Figure 5 (A) Effect of co-administration of carvedilol (CAR) on chronic haloperidol (HPD) mediated depletion in the level of forebrain antioxidant enzyme catalase. Values expressed as per cent response of vehicle treated control group. Each value represents mean value of six animals. $^{a}P<0.05$ as compared to vehicle treated control group. $^{b}P<0.05$ as compared to haloperidol treated group (ANOVA followed by Dunnett's test). (B) Effect of co-administration of carvedilol (CAR) on chronic chlorpromazine (CPZ) mediated depletion in the level of forebrain antioxidant enzyme catalase. Values expressed as per cent response of vehicle treated control group. Each value represents mean value of six animals. $^{a}P<0.05$ as compared to vehicle treated control group. $^{b}P<0.05$ as compared to chlorpromazine treated group (ANOVA followed by Dunnett's test).

metabolism of catecholamines) is an integral part of this cascade. Thus, the possibility exists of a direct interaction with specific membrane components. Cohen & Zubenko (1985) have in fact demonstrated that striatal cell membranes of rats chronically treated with neuroleptics exhibit abnormal physicochemical properties. It is conceivable that the changes in membrane properties may be related to free radical production. In addition, chlorpromazine causes an increase in the level of brain manganese, which in turn may potentiate the damage caused by free radicals (Weiner *et al*., 1980).

Another possibility is that neuroleptics suppress the activity of certain detoxifying enzymes, leaving cells unprotected especially if basal enzyme activity is low or the free radical-scavenging mechanisms are less effective. Free radicals are highly reactive with specific cellular components and have cytotoxic properties (Ravindranath & Reed, 1990), and neuronal loss in the striatum has been reported in animals treated chronically with neuroleptics (Nielsen & Lyon, 1978). Neuroleptics may also have direct cytotoxic effect *via* the production of toxic metabolites (Gorrod & Fang, 1993; Wright *et al*., 1998). Reduced haloperidol is oxidized to a pyridinium metabolite (RHPP+) in blood and brain (Eyles *et al*., 1996), which is also thought to be a mitochondrial toxin. As reduced haloperidol concentrations are $\sim$5 times higher in the elderly (Chang *et al*., 1996) this could contribute to their predisposition to develop TD. Treatment with structurally related pyridinium species induces VCMs in baboons (Halliday *et al*., 1999) and a reduction in neuronal volume in the basal forebrain and hypothalamus. Galili *et al*. (2000) reported that the direct neurotoxic effects of haloperidol and its metabolites on mouse neuronal cultures and PC-12 cells were reversed by antioxidants. Burkhardt *et al*. (1993) reported that, like MPP^{+}, the metabolites of haloperidol, chlorpromazine, and thioxanthine inhibited complex I of the electron transport chain, clozapine was also found to inhibit complex I, but at a much higher concentration, this might be one of the possible mechanisms for the development of TD.

In the present study chronic haloperidol or chlorpromazine but not clozapine treated animals showed decreased levels of glutathione and increased levels of lipid peroxidation products as compared to vehicle treated control animals.

Chronic haloperidol, or chlorpromazine but not clozapine treated animals showed low levels of detoxifying enzymes such as SOD and catalase as compared to vehicle treated control animals suggesting possible induction of free radical generation by chronic haloperidol and chlorpromazine treatment. Carvedilol dose dependently decreased the elevated level of lipid peroxidation products in haloperidol or chlorpromazine treated animals, elevated the cellular defence mechanisms such as glutathione, and also induced the production of SOD and catalase, further suggesting the role of free radical in the pathophysiology of haloperidol or chlorpromazine-induced orofacial dyskinesia and possible antioxidant action of carvedilol.

Carvedilol inhibited lipid peroxidation in myocardial cell membranes initiated by oxygen radicals generated by chemical, enzymatic or cellular systems (Yue *et al*., 1992b), also protected endothelial cells from oxygen radical-mediated injury. Carvedilol inhibited superoxide ion release from activated neutrophils (Yue *et al*., 1992c). Carvedilol preserves the endogenous antioxidant systems (i.e., vitamin E and glutathione) that are normally consumed when tissues or organs are exposed to oxidative stress (Lysko *et al*., 1995), and protects neuronal cells from injury induced by oxygen free radicals *in vitro* and from ischemia and reperfusion damage *in vivo* (Lysko *et al*., 1992). Carvedilol also protects against peroxynitrite ($ONOO^-$) toxicity and reported to increase glutathione levels (Feuerstein *et al*., 1997).

The antioxidant action of carvedilol has been speculated to be due to (1) inhibition of direct cytotoxic actions of free radicals, (2) prevention of oxygen free radicals from activating transcription factors such as NF-κB and (3) protection and replenishing the endogenous antioxidant defence mechanisms, glutathione and vitamin E. However, the dynamics of the antioxidant action of carvedilol are not known. Yue *et al*. (1992a) have reported that carvedilol inhibits lipid peroxidation by scavenging free radicals, while some others reported that carvedilol is not free radical scavenger but rather sequester of ferric ion (Tadolini & Franconi, 1998; Noguchi *et al*., 2000). Carvedilol is also reported to act as a calcium channel blocker (Nichols *et al*., 1989; Ruffolo *et al*., 1990). Calcium having a role in the pathophysiology of haloperidol-induced oral dyskinesia (Naidu & Kulkarni, 2001) the beneficial effects of carvedilol might be due to its calcium channel blocking activity, this has to be further confirmed.

Whatever could be the mechanism our results confirm that carvedilol has an antioxidant activity. This activity appears particularly relevant for the understanding of the molecular mechanisms that underlie the action of carvedilol, but also represents a valid rational for the use of carvedilol in the prevention and therapy of neuroleptic-induced orofacial dyskinesia.

In conclusion the findings of the present study strongly suggest the role of oxidative stress in the pathophysiology of neuroleptic-induced orofacial dyskinesia and that carvedilol could be used for the prevention or treatment of neuroleptic-induced orofacial dyskinesia.

Financial support provided by SUN Pharmaceuticals Ltd., Mumbai and technical support by Ms Saraswati Gupta is gratefully acknowledged.

References

ABREU, R.M.V., SANTOS, D.J.S.L. & MORENO, A.J.M. (2000). Effects of carvedilol and its analog BM-910228 on Mitochondrial function and oxidative stress. *J. Pharmacol. Exp. Ther.,* **295,** 1022–1030.

ANDREASSEN, O.A. & JORGENSEN, H.A. (2000). Neurotoxicity associated with neuroleptic induced oral dyskinesias in rats implications for tardive dyskinesia? *Prog. Neurobiol.,* **61,** 525–541.

BALIJEPALLI, S., KENCHAPPA, R.S., BOYD, M.R. & RAVINDRANATH, V. (2001). Protein thiol oxidation by haloperidol results in inhibition of mitochondrial complex I in brain regions: comparison with atypical antipsychotics. *Neurochem. Int.,* **38,** 425–435.

BURKHARDT, C., KELLY, J.P., LIM, Y.H., FILLEY, C.M. & PARKER, W.D.J. (1993). Neuroleptic medications inhibit complex I of the electron transport chain. *Ann. Neurol.,* **33,** 512–517.

CADET, J.L., LOHR, J.B. & JESTE, D.V. (1986). Free radicals and tardive dyskinesia. *Trends Neurosci.,* **9,** 107–108.

CADET, J.L., LOHR, J.B. & JESTE, D.V. (1987). Tardive dyskinesia and schizophrenic burnout: the possible involvement of cytotoxic free radicals. In *Handbook of Schizophrenia: The Neurochemistry and Pharmacology of Schizophrenia*. ed. Henn, F.A. & DeLisi, L.E. pp. 425–438. Amsterdam: Elsevier.

CASEY, D.E. (2000). Tardive dyskinesia: Pathophysiology and Animal Models. *J. Clin. Psychiat.,* **61,** 5–9.

CHANG, W.H., JANN, M.W., CHIANG, T.S., LIN, H.N., HU, W.H. & CHIEN, C.P. (1996). Plasma haloperidol and reduced haloperidol concentrations in a geriatric population. *Neuropsychobiol.,* **33,** 12–16.

COHEN, G. & SPINA, M.B. (1988). Hydrogen production in neurones: Implications for understanding Parkinson's disease. In: *Progress in Parkinson's Research*. ed. Hefti, F. & Weiner, W.J. pp. 119–126. New York: Plenum.

COHEN, G. & ZUBENKO, G.S. (1985). In vivo effects of psychotropic agents on the physical properties of cell membranes in the rat brain. *Psychopharmacology,* **86,** 365–368.

COYLE, J.T. & PUTTFARCKEN, P. (1993). Oxidative stress glutamate and neurodegenerative disorders. *Science,* **262,** 689–695.

CREESE, G., BURT, D. & SYNDER, S.I.L. (1976). Dopamine receptor binding and pharmacological potencies and antischizophrenic drugs. *Science,* **192,** 481–483.

EGAN, M.F., APUD, J. & WYATT, R.J. (1997). Treatment of tardive dyskinesia. *Schizophr. Bull.,* **23,** 583–609.

ELKASHEF, A.M. & WYATT, R.J. (1999). Tardive dyskinesia: possible involvement of free radical and treatment with vitamin E. *Schizophr. Bull.,* **25,** 731–740.

ELLMAN, G.L. (1959). Tissue sulfhydryl groups. *Arch. Biochem. Biophys.,* **82,** 70–77.

EYLES, D.W., MCGRATH, J.J. & POND, S.M. (1996). Formation of pyridinium species of haloperidol in human liver and brain. *Psychopharmacol.,* **125,** 214–219.

FEUERSTEIN, G.Z., SHUSTERMAN, N.H. & RUFFOLO JR., R.R. (1997). Carvedilol update IV: prevention of oxidative stress, cardiac remodeling and progression of congestive heart failure. *Drugs of Today,* **33,** 453–457.

FEUERSTEIN, R. & YUE, T.-L. (1994). Carvedilol: A novel multiple action antihypertensive drug that provides major organ protection. *Cardiovasc. Drug Rev.,* **12,** 85–104.

FIBIGER, H.C. & LLOYD, K.G. (1984). Neurobiological substrates of tardive dyskinesia: the GABA hypothesis. *Trends Neurol. Sci.,* **7,** 462–464.

FLECKENSTEIN, A.E., METZGER, R.R., BEYELER, M.L., GIBB, J.W. & HANSON, G.R. (1997). Oxygen radicals diminish dopamine transporter function in rat striatum. *Eur. J. Pharmacol.,* **334,** 111–114.

GALILI, R., MOSBERG, GIL-AD, W., WEIZMAN, A., MELAMED, E. & OFFEN, D. (2000). Haloperidol-induced neurotoxicity – possible implications for tardive dyskinesia. *J. Neural. Transm.,* **107,** 479 – 490.

GORROD, J.W. & FANG, J. (1993). On the metabolism of haloperidol. *Xenobiotica,* **23,** 495 – 508.

GROHMAN, R., KOCH, R. & SCHMIDT, L.G. (1990). Extrapyramidal symptoms in neuroleptic recipients. In *Adverse Drug Reactions*. pp. 71 – 82. Basel: Birkhauser Verlag.

GUPTA, S., MOSNIK, D., BLACK, D.W., BERRY, S. & MASAND, P.S. (1999). Tardive dyskinesia: review of treatments Past, Present, and future. *Ann. Clin. Psychiat.,* **11,** 257 – 266.

HALLIDAY, G.M., POND, S.M., CARTWRIGHT, H., MCRITCHIE, D.D., CASTAGNOLIN, JR. N. & VAN DER SCHYF, C.J. (1999). Clinical and neuropathological abnormalities in baboons treated with HPTP, the tetrohydropyridine analogue of haloperidol. *Exp. Neurol.,* **158,** 155 – 163.

KONO, Y. (1978). Generation of superoxide radical during auto-oxidation of hydroxylamine and an assay for superoxide dismutase. *Arch. Biochem. Biophy.,* **186,** 189 – 195.

KULKARNI, S.K. & NAIDU, P.S. (2001). Tardive dyskinesia: An update. *Drugs of Today,* **37,** 97 – 119.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin-Phenol reagent. *J. Biol. Chem.,* **193,** 262 – 275.

LUCK, H. (1971). Catalase. In *Methods of Enzymatic Analysis*. ed. Bergmeyer, H.U. pp. 885 – 893. New York: Academic Press.

LYSKO, P.G., FEUERSTEIN, G.Z. & RUFFOLO, JR. R.R. (1995). Carvedilol: A novel multiple action antihypertensive drug. *Pharm. News,* **2,** 12 – 16.

LYSKO, P.G., LYSKO, K.A., YUE, T.-L., WEBB, C.L., GU, J.-L. & FEUERSTEIN, G.Z. (1992). Neuroprotective effects of carvedilol, a new antihypertensive agent, in cultured rat cerebellar neurons and in gerbil global brain ischemia. *Stroke,* **23,** 1630 – 1636.

LYSKO, P.G., WEBB, C.L., GU, J.-L., OHLSTEIN, E.H., RUFFOLO, JR. R.R. & YUE, T.-L. (2000). A comparison of carvedilol and metoprolol antioxidant activities in vitro. *J. Cardiovas. Pharmacol.,* **36,** 277 – 281.

MA, X.-L., YUE, T.-L., LOPEZ, B.L., BARONE, F.C., CHRISTOPHER, T.A., RUFFOLO, JR. R.R. & FEUERSTEIN, G.Z. (1996). Carvedilol, a new beta adrenoceptor blocker and free radical scavenger, attenuates myocardial ischemia reperfusion injury in hypercholesterolemic rabbits. *J. Pharmacol. Exp. Ther.,* **277,** 128 – 136.

NAIDU, P.S. & KULKARNI, S.K. (2001). Excitatory mechanisms in neuroleptic-induced vacuous chewing movements (VCMs): Possible involvement of calcium and nitric oxide. *Behav. Pharmacol.,* **12,** 209 – 216.

NAIDU, P.S. & KULKARNI, S.K. (2001a). Effect of 5-HT1A and 5-HT2A/2C receptor modulations on neuroleptic-induced vacuous chewing movements. *Eur. J. Pharmacol.,* **428,** 81 – 86.

NAIDU, P.S. & KULKARNI, S.K. (2001b). Possible involvement of prostaglandins in haloperidol-induced orofacial dyskinesia in rats. *Eur. J. Pharmacol.,* **430,** 295 – 298.

NICHOLS, A.J., SULPIZIO, A.C., ASHTON, D.J., HIEBLE, J.P. & RUFFOLO, JR. R.R. (1989). In vitro pharmacological profile of the novel β-adrenoceptor antagonist and vasodilator carvedilol. *Pharmacology,* **39,** 327 – 336.

NIELSEN, E.B. & LYON, M. (1978). Evidence for cell loss in corpus striatum after long-term treatment with a neuroleptic drug (flupenthixol) in rats. *Psychopharmacology,* **59,** 85 – 89.

NOGUCHI, N., NISHINO, K. & NIKI, E. (2000). Antioxidant action of the antihypertensive drug carvedilol, against lipid peroxidation. *Biochem. Pharmacol.,* **59,** 10669 – 1076.

RABASSEDA, X. (1998). Carvedilol: an effective antihypertensive drug with antiischemic/antioxidant cardioprotective properties. *Drugs of Today,* **34,** 905 – 926.

RAVINDRANATH, V. & REED, D.J. (1990). Glutathione depletion and formation of glutathione protein mixed disulfide following exposure of brain mitochondria to oxidative stress. *Biochem. Biophys. Res. Commun.,* **169,** 150 – 158.

RICHARDSON, M.A., HAUGLAND, G., PASS, R. & CRAIG, T.J. (1986). The prevalence of tardive dyskinesia in a mentally retarded population. *Psychopharmacol. Bull.,* **22,** 243 – 249.

RUFFOLO JR., R.R., GELLAI, M., HIEBLE, J.P., WILLWTTE, R.N. & NICHOLS, A.J. (1990). The Pharmacology of carvedilol. *Eur. J. Clin. Pharmacol.,* **38**(suppl): S82 – S88.

SAGARA, Y. (1998). Induction of reactive oxygen species in neurones by haloperidol. *J. Neurochem.,* **71,** 1002 – 1012.

SASAKI, H., HASHIMOTO, K., MAEDA, Y., INADA, T., KITAO, Y., FUKUI, S. & IYO, M. (1995). Rolipram, a selective c-AMP phosphodiesterase inhibitor suppresses oro-facial dyskinetic movements in rats. *Life Sci.,* **56,** 443 – 447.

SHIVAKUMAR, B.R. & RAVINDRANATH, V. (1993). Oxidative stress and thiol modification induced by chronic administration of haloperidol. *J. Pharmacol. Exp. Ther.,* **265,** 1137 – 1141.

SOARES, K.V. & MCGRATH, J.J. (2000). Vitamin E for neuroleptic-induced tardive dyskinesia. *Cochrane Database Syst. Rev.,* **2,** CD000209.

TADOLINI, B. & FRANCONI, F. (1998). Carvedilol inhbition of lipid peroxidation. A new antioxidative metabolism. *Free Radic. Res.,* **29,** 377 – 387.

VAIRETTI, M., FELETTI, F., BATTAGLIA, A., PAMPARANA, F., CANONICO, P.L., RICHELMI, P. & BERTI, F. (1999). Haloperidol-induced changes in glutathione and energy metabolism effect of nicergolin. *Eur. J. Pharmacol.,* **367,** 67 – 72.

WEINER, W.J., NAUSIEDA, P.A. & KLAWANS, H.L. (1980). Regional brain manganese levels in animal model of tardive dyskinesia. In *Tardive Dyskinesia Research and Treatment*. ed. Fann, W.E., Smith, R.C., Davis, J.M. & Domino, E.F. pp. 159 – 163. New York: SP Medical and Sciences Books.

WILLS, E.D. (1966). Mechanism of lipid peroxide formation in animal tissues. *Biochem. J.,* **99,** 667 – 676.

WRIGHT, A.M., BEMPONG, J., KIRBY, M.L., BARLOW, R.L. & BLOOMQUIST, J.R. (1998). Effects of haloperidol metabolites on neurotransmitter uptake and release: possible role of neurotoxicity and tardive dyskinesia. *Brain Res.,* **788,** 215 – 222.

YOSHIOKA, T., IWAMOTO, N., TSUKAHARA, F., IRIE, K., URAKAWA, I. & MURAKI, T. (2000). Anti-NO action of carvedilol in cell-free system and in vascular endothelial cells. *Br. J. Pharmacol.,* **129,** 1530 – 1535.

YUE, T.-L., CHENG, H.-Y., LYSKO, P.G., MCKENNA, P.J., FEUERSTEIN, R., GU, J.-L., LYSKO, K.A., DAVIS, L.L. & FEUERSTEIN, G.Z. (1992a). Carvedilol, a new vasodilator and β-adrenergic blocking agents adrenoceptor antagonist, is an antioxidant and free radical scavenger. *J. Pharmacol. Exp. Ther.,* **263,** 92 – 98.

YUE, T.-L., LIU, T. & FEUERSTEIN, G. (1992b). Carvedilol, a new vasodilator and β-adrenoreceptor antagonist, inhibits oxygen-radical-mediated lipid peroxidation in swine ventricular membranes. *Pharmacol. Commun.,* **1,** 27 – 35.

YUE, T.-L., MCKENNA, P.J., RUFFOLO JR., R.R. & FEUERSTEIN, G.Z. (1992c). Carvedilol, a new β-adrenoreceptor antagonist and vasodilator antihypertensive drug, inhibits superoxide release from human neutrophils. *Eur. J. Pharmacol.,* **214,** 277 – 280.

YUE, T.-L., WANG, X., GU, J.-L., RUFFOLO JR., R.R. & FEUERSTEIN, G.Z. (1995). Carvedilol, a new vasodilating β-adrenergic blocker, inhibits oxidation of low-density lipoproteins by vascular smooth muscle cells and prevents leukocyte adhesion to smooth cells. *J. Pharmacol. Exp. Ther.,* **273,** 1442 – 1449.

(Received January 29, 2002
Revised March 7, 2002
Accepted March 12, 2002)