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Effect of piribedil and its metabolite, S584, on brain lipid peroxidation in vitro and in vivo

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Abstract

We studied the effect of piribedil (1-3,4-methylendioxybenzyl-4-(2-pyrimidyl) piperazine) and its catechol metabolite, S584 (1-(3,4-dihydroxybenzyl-4-(2-pyrimidinyl)-piperazine), on rat brain lipid peroxidation (a) in vitro in rat synaptosomes and cortical slices after induction of an oxidative stress and (b) in vivo in mouse brain after short-term exposure (two and three 4-h cycles) to O_2/CO_2 (95%:5%). The metabolite (10^{-4}–10^{-5} M), but not piribedil, prevented Fe^{3+}-stimulated lipid peroxidation in rat synaptosomes and in rat cortical slices incubated with high oxygen concentrations. Piribedil (7.5 and 30 mg/kg, orally), counteracted the increase in thiobarbituric reactive substances in the brain of mice only when these were exposed to two or three cycles of a high oxygen concentration. S584 (30 mg/kg, orally) reduced thiobarbituric acid reactive substances in brain in mice exposed either to air (control) or to three cycles of a high oxygen concentration. These results suggest that piribedil has an antiperoxidative effect in brain, which may be partly related to the in vivo formation of the catechol metabolite, S584. © 1997 Elsevier Science B.V.

Keywords: Piribedil; Lipid peroxidation; Brain; Oxygen exposure, high

1. Introduction

Piribedil (1-3,4-methylendioxybenzyl-4-(2-pyrimidyl) piperazine, Trivastal®) induces in animals behavioral and neurochemical changes indicative of a direct postsynaptic action on dopamine receptors (Corrodi et al., 1972; Costall and Naylor, 1973, Consolo et al., 1975; Hall et al., 1983). The drug is considered an agonist of the dopamine D_2 receptors, but may also have affinity for the D_3 subtype (Cagnotto et al., 1996). It has been used as an anti-Parkinson agent and in the treatment of other clinical disorders involving dysfunction of the dopaminergic system (Dourish, 1983; Jenner, 1992).

Piribedil reverses free radical production in the gerbil brain after cerebral ischemia (Delbarre et al., 1995). Dopamine, aspartate, glutamate and gamma-aminobutyric acid alterations are also counteracted and some protection was observed against neurological signs in brain ischemia. Whether and if so, how these effects are related to the dopaminergic properties of piribedil remains to be clarified.

The drug undergoes rapid and extensive biotransformation in man and animals and several hydroxylated and N-oxide metabolites have been identified in body fluids, with quantitative differences between species (Campbell et al., 1973). The catechol metabolite, 1-(3,4-dihydroxybenzyl-4-(2-pyrimidinyl)-piperazine (S584), induces dopamine-like effects in rats, although brain concentration–response studies suggest that it does not contribute to the dopaminergic effects of the parent compound in this species (Sarati et al., 1991). However, it is reported to have slight antioxidant activity in rat synaptosomes, similarly to adrenaline, noradrenaline and dopamine (Gilbert and Sawas, 1983).

To further characterize the antioxidant activity of piribedil we investigated its effects on the production of thiobarbituric acid reactive substances as an expression of lipid peroxidation (a) in vitro in rat synaptosomes and brain cortical slices after induction of an oxidative stress, and (b) in vivo in mouse brain after exposure to a high oxygen concentration. The activity of the catechol metabolite was also studied in these models. Brain concentrations of S584 were compared after pharmacologically effective doses of the two compounds to see whether the parent drug's antioxidant activity depends on the formation of its catechol metabolite.

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2. Materials and methods

2.1. Animals and drugs

Piribedil monomethanesulphonate and S584 were kindly supplied by Laboratoires Servier, Gidy. Other reagents were of analytical grade.

Male Crl:CD(SD)BR rats (Charles River, Italy) weighing about 200 g and female mice weighing about 20 g at the start of the study were used. Procedures involving animal and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., Suppl. 40, 18 Febbraio 1992, Circolare No. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987; Guide for Care and Use of laboratory Animals, US National Research Council, 1996).

2.2. In vitro studies

A crude fraction of rat brain synaptosomes (P2 pellets) was prepared according to Dodd et al. (1981). Lipid peroxidation was induced as described by Gilbert and Sawas (1983). Briefly, approximately 1 mg protein of P2 pellets was added to 50 mM imidazole/HCl buffer, pH 7.4, containing 150 mM NaCl, 10 mM KCl, with and without the addition of various concentrations of the compounds (10^{-6} to 10^{-4} M), dissolved in a lightly acidic solution to prevent autooxidation, and incubated for 30 min at 37°C. In some experiments Fe^{3+}/ADP (1:33) + 250 μM ascorbic acid, according to the method described by Zaleska et al. (1989) was added to the incubation medium in order to stimulate lipid peroxidation.

Reactions were stopped by the addition of 50 μl 1.6% hydroquinone in acetic acid; then thiobarbituric reactive substances were measured according to Wilbur et al. (1949), with the modification described by Kikugawa et al. (1992), i.e., samples were kept in ice for exactly 60 min and only afterwards were they placed in a boiling water bath for 60 min. This resulted in less variability within the experiments.

The antioxidant effect of S584 and piribedil was also evaluated in rat brain slices incubated in a high oxygen concentration. Approximately 150 mg of brain slices (350 μm thick, prepared using a McIlwain chopper) were incubated in Krebs–Ringer phosphate buffer containing 120 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgCl_2$, 16 mM sodium phosphate (pH 7.4), and 10 mM glucose (Kovachich and Mishra, 1980). The medium was bubbled with O_2/CO_2 (95%:5%) for 15 min before use. After the addition of S584 or piribedil (10^{-4} M), flasks containing samples were capped and placed in a water bath at 37°C. At the beginning of the experiment and after 30 min incubation, the flasks were saturated with O_2/CO_2 (95%:5%), or air under normobaric conditions. Reactions were stopped with cold 20% trichloroacetic acid after 60 min incubation. The thiobarbituric-reactive substance content was measured as above.

2.3. In vivo studies

To evaluate the effect of piribedil and S584 on H_2O_2 production and lipid peroxidation in vivo in brain, mice were exposed to a high oxygen concentration (O_2/CO_2, 95%:5%, in normobaric conditions) in a 30 × 30 × 30 cm cage for 4 h per day, and killed under light ether anesthesia after two or three exposures. Piribedil (7.5 and 30 mg/kg, orally) and S584 (30 mg/kg, orally) were administered 1 h before each exposure and 8 h later every day. The brains were rapidly excised and homogenized in 1.5 ml 50 mM buffer phosphate with 0.1 mM EDTA and 0.1% Triton X 100, pH 7.0. Aliquots were used for measuring thiobarbituric-reactive substances after protein precipitation with 10% trichloroacetic acid. The assay was done as described above.

In initial studies mice were killed just before each cycle to measure brain concentrations of piribedil and its active metabolite. Because these brain concentrations were below the limit of sensitivity of the analytical procedure, further groups of mice were given the drugs at the higher dose and were killed at various times for plasma and tissue assays of the two compounds. Piribedil and S584 plasma and tissue concentrations were determined with slight modifications of the high performance liquid chromatographic method with solid-phase extraction, as previously described (Sarati and Caccia, 1992).

The area under the curve (AUC_t) for the sampling interval was determined with the trapezoidal method. The observed maximum concentration (C_{max}) and the time of its occurrence (t_{max}) were taken directly from the data. Because of the limited number of experimental points other conventional kinetic parameters were not included.

2.4. Statistical analysis

One-way analysis of variance was performed and when '*F*' was significant, Dunnett's or Tukey's tests were applied for multiple comparisons.

3. Results

The activity of piribedil and its metabolite, S584, was tested in vitro on rat synaptosomes. S584 (10^{-4} and 10^{-5} M) reduced the formation of thiobarbituric reactive substances by about 75% and 80%, respectively, compared to the control value for synaptosomes incubated under basal conditions (Fig. 1). When thiobarbituric-reactive substance production was stimulated by Fe^{3+}/ADP + ascorbic acid, S584 was active only at 10^{-4} M. Piribedil was not active under either basal or stimulated conditions (data not shown).

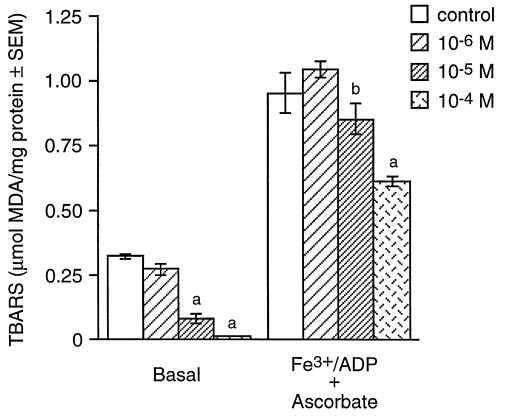

Fig. 1. Effect of S584 on thiobarbituric reactive substances (expressed as μmol MDA/mg protein ± S.E.M.) in the absence and presence of Fe^{3+}/ADP (1:33) + 250 μM ascorbic acid in crude synaptosomal fractions of rat brains. Each value is the mean of 3–4 replications from one representative experiment. At least two experiments were done. [a] $P < 0.01$ in comparison to control; [b] $P < 0.05$ in comparison to control.

Lipid peroxidation was measured in rat cortical slices exposed to a high oxygen concentration (O_2/CO_2, 95%:5%, for 60 min). This treatment increased thiobarbituric reactive substances by 26% in the absence of drugs; the addition of 10^{-4} M S584 (Table 1) but not of its parent drug counteracted this effect.

To evaluate the effect of piribedil and S584 on brain lipid peroxidation in vivo, mice were exposed to O_2/CO_2 (95%:5%) in cycles of 4 h per day. The brain content of thiobarbituric-reactive substances was measured after two and three cycles (Table 2). The basal levels of brain thiobarbituric-reactive substances were different in various experiments, but the increase after exposure to oxygen always ranged within 40–50% in comparison to air. After three exposures the increase amounted to 41%. Piribedil twice a day (1 h before oxygen exposure and 8 h later), at the dose of 7.5 mg/kg and 30 mg/kg, reduced thiobarbituric acid-reactive substance production to control levels in mice exposed twice. In mice exposed three times only the dose of 30 mg/kg was active. The metabolite, S584 (30 mg/kg), counteracted the rise in thiobarbituric-reactive substances induced by two and three exposures to high oxygen concentrations and significantly lowered thiobarbituric reactive substances in control mice exposed to air.

Table 1
Effect of piribedil and S584 on thiobarbituric reactive substance (TBARS) formation (expressed as mean μmol MDA/g fresh tissue ± S.E.M.) induced in rat brain slices by exposure to a high oxygen concentration

Drugs	*M*	TBARS produced in brain slices/60 min μmol MDA/g fresh tissue (mean ± S.E.M.)	
		air	O_2/CO_2
Control		27.4 ± 1.66	34.7 ± 1.04 [a]
Piribedil	$10-4$	27.8 ± 2.25	34.2 ± 1.15 [a]
S584	$10-4$	28.3 ± 1.69	28.2 ± 2.22 [b]

Each value is the mean for 6–7 samples run in duplicate.
[a] $P < 0.01$ in comparison to control.
[b] $P < 0.05$ in comparison to O_2/CO_2.

Table 2
Effect of piribedil (7.5 and 30 mg/kg, orally) and S584 (30 mg/kg, orally) on thiobarbituric reactive substance (TBARS) formation (expressed as mean μmol MDA/g fresh tissue ± S.E.M.) induced in mouse brain by exposure to a high oxygen concentration in vivo

	Treatment		TBARS produced μmol MDA/g brain (mean ± S.E.M.)	
		mg/kg	2 exposures	3 exposures
Air		—	61.8 ± 3.5	43.4 ± 5.5
Air	piribedil	7.5	58.2 ± 3.8	43.0 ± 1.4
Air	piribedil	30	66.3 ± 2.1	41.7 ± 8.4
O_2/CO_2		—	92.3 ± 9.9 [a]	61.5 ± 2.8 [a]
O_2/CO_2	piribedil	7.5	65.4 ± 3.0 [b]	54.2 ± 4.3
O_2/CO_2	piribedil	30	63.2 ± 5.3 [b]	50.8 ± 2.6 [c]
Air		—	61.9 ± 3.4	54.8 ± 1.1
Air	S584	30	43.3 ± 2.0 [a]	44.5 + 1.7 [a]
O_2/CO_2		—	95.2 ± 6.4 [a]	74.8 ± 2.8 [a]
O_2/CO_2	S584	30	56.5 ± 4.1 [b]	60.4 ± 3.8 [c]

Each value is the mean for 5–6 mice.
[a] $P < 0.01$ in comparison to control with air.
[b] $P < 0.01$ in comparison to control with O_2/CO_2.
[c] $P < 0.05$ in comparison to control with O_2/CO_2.

Brain concentrations of the parent drug and its catechol metabolite measured just before each cycle (i.e., 1 h after dosing) were below the limit of sensitivity of the analytical procedure (0.2 nmol/g, using approximately 0.4 g of brain tissue).

To clarify their kinetics, piribedil and S584 were given orally at 30 mg/kg and the animals were killed at short times after treatment. Piribedil appeared rapidly in plasma (Fig. 2A), but the mean C_{max} were low and variable (1.6 ± 1.2 μM at 5 min) and rapidly declined to levels close to the limits of analytical sensitivity within a few minutes after treatment. These concentrations were largely exceeded by those of the metabolite, S584, which reached a mean C_{max} of 10.2 ± 8.3 μM within 5 min and then decreased more slowly than that of the parent drug, although the elimination $t_{1/2}$ could not be determined because of the shortage of experimental points. S584 given as such gave a mean plasma concentration profile almost parallel to that after piribedil, with similar, although variable, concentrations within 60 min of treatment (Fig. 2). Its mean plasma C_{max} and AUC also were similar to those after piribedil.

In brain, piribedil reached concentrations comparable to those in plasma within the first 15 min of dosing (Fig. 2B). As in plasma, these concentrations were variable and short-lasting. Mean brain concentrations of the metabolite were 12–14 times lower than those in plasma after piribedil, and tissue-to-plasma ratios were similar after S584. This was probably related to the poor blood–brain barrier per-

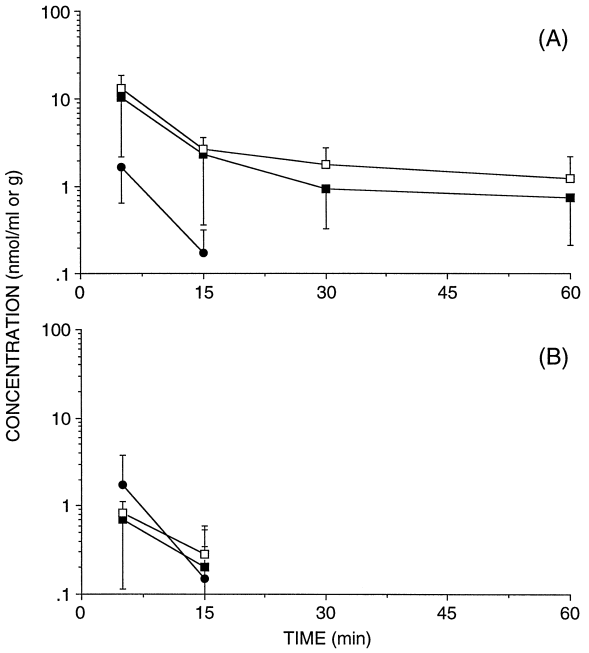

Fig. 2. Pharmacokinetics of piribedil and S584 in mouse plasma and brain. Mean plasma (A) and brain (B) concentration–time curves of piribedil (closed circles) and S584 (closed squares) after piribedil (30 mg/kg, orally). Open squares indicate the plasma and brain concentrations of S584 (30 mg/kg, orally). Each value is the mean ± S.D. for 4–5 mice.

meability of the metabolite because its tissue-to-plasma ratio in other tissues, such as the lung, approached unity at all times (data not shown). Again, however, the mean brain concentration–time profile of the metabolite was almost parallel for the two experimental conditions, with mean brain C_{max} amounting to less than 1 nmol/g at 5 min then rapidly declining to levels close to the limits of sensitivity after either piribedil or S584.

4. Discussion

Dopamine may have a dual effect on lipid peroxidation: at physiological concentrations it inhibits basal and Fe^{3+}-stimulated lipid peroxidation, but increases oxygen radical species and lipid peroxidation under conditions of stimulated release, especially in the presence of high Fe^{3+} (Ben-Shachar and Youdim, 1993). Presynaptic dopamine D_2 receptors can modulate dopamine release and uptake, providing less neurotransmitter and reducing the production of free radicals (Kim et al., 1980; Kornhuber and Kornhuber, 1986). Dopamine D_2 receptors appear to be located on glutamate and aspartate terminals of the corticostriatal pathway, possibly providing a signal to inhibit the release of excitatory amino acids implicated in the pathogenesis of cerebral ischemia (Maura et al., 1988, 1989). Dopamine D_3 receptors have been described recently which may act as autoceptors, but their functional significance has not yet been clarified (Sokoloff et al., 1990).

Piribedil is considered an agonist of the dopamine D_2 receptors, although recent studies show that it binds at dopamine D_3 sites with comparable (Millan et al., 1995) or even greater affinity (Cagnotto et al., 1996). It is reported to reverse the increase of dopamine, excitatory amino acids and free radicals and to reduce lipid peroxidation in brain tissue after ischemia-reperfusion induced injury in gerbils (Delbarre et al., 1995). It also protects the pig cochlea from transient ischemic damage, a system where dopamine seems to be involved in postsynaptic regulation of hair cells (D'Aldin et al., 1995).

The present study confirms and extends these findings, showing that the drug has antioxidant activity in an in vivo mouse model of mild oxidative stress, in which animals are exposed to cycles of a high oxygen concentration. The mechanism(s) of this action, however, cannot be readily explained, although the present results raise doubts about the direct involvement of the unchanged compound in its antioxidant effects in vivo in mice.

After a pharmacologically effective dose of 30 mg/kg, piribedil appears to undergo rapid and extensive biotransformation, resulting in very low plasma concentrations. Not only is this not surprising since a similar pharmacokinetic profile has been observed in all the species investigated so far (Campbell et al., 1973; Sarati et al., 1991), but it is consistent with its lipophylic characteristics which make piribedil a first-pass rapidly cleared drug.

The brain concentration–time profile paralleled that in plasma, confirming that piribedil rapidly and freely distributes across the blood/brain barrier (Sarati et al., 1991). As shown in Fig. 2 an oral dose of 30 mg/kg produced brain concentrations of unchanged drug of about 2 μM, in terms of C_{max}. Even though the brain concentrations rapidly fell below the limit of sensitivity of the analytical procedure, the amount of piribedil in the brain during each cycle of oxygen exposure might still have been sufficient to act at dopamine D_2 receptors because the drug's affinity for these sites is only 0.1–1 μM, depending on the species and tissue expressing these receptors (Millan et al., 1995; Cagnotto et al., 1996). Moreover, specific localizations of piribedil cannot be excluded, as its subcellular distribution is not known. Similarly an effect on dopamine D_3 receptors cannot be excluded. In fact, piribedil affinity may be even higher for dopamine D_3 receptors than for D_2 receptors, but little is known about the functional significance of the dopamine D_3 receptors. These observations suggest that the antioxidant effect of piribedil might be due, at least in part, to its dopamine D_2 receptor agonist action.

However, piribedil had no antioxidant activity in vitro in rat synaptosomes and brain cortical slices after induction of an oxidant stress and in vivo under basal conditions. In the presence of an oxidative stress in vivo, piribedil counteracted the rise of thiobarbituric reactive substance production. Its catechol derivative, S584, signifi-

cantly reduced thiobarbituric reactive substance production and lipid peroxidation in vitro, and, in vivo in mouse brain, prevented the increase of thiobarbituric-reactive substances both under basal conditions and after high oxygen exposure. These findings support the hypothesis that the antioxidant activity of piribedil in vivo in the mouse is mediated by its catechol metabolite, at least under conditions of oxidative stress. Unlike piribedil, however, S584 shows very low affinity for dopamine D_2 sites (T. Mennini, personal communication), but a significant effect on the dopamine D_1 receptor subtype (Miller and Iversen, 1974). A putative neuroprotective action of dopamine receptor agonists has recently been speculated upon (Jenner, 1995, Wolters et al., 1995). Why piribedil is active via its metabolite only in the presence of an oxidative stress is not yet clear. This may depend on a different distribution of the metabolite or on an altered susceptibility of Na^+,K^+-ATPase under these abnormal conditions. In fact, it was reported that S584, like other catechols, stimulates ATPase activity, which inversely correlates with lipid peroxidability (Sawas and Gilbert, 1982).

Further evidence of the role of S584 in mediating the in vivo antioxidant effect of piribedil arises from the pharmacokinetic study. Plasma concentrations of the metabolite after oral piribedil were always higher than those of the parent drug, confirming that demethylation of the methylenedioxyphenyl bridge is a main route of piribedil metabolism in this species (Campbell et al., 1973).

Brain concentrations of the metabolite were similar to those of the parent compound, because the polar metabolite enters the brain poorly, as already observed in rat (Sarati et al., 1991). Although these in vivo concentrations were much lower than those used to obtain antiperoxidative activity in vitro, they were comparable to those reached after pharmacologically effective doses of S584 as such. It should also be considered that the sensitivity of the in vitro and in vivo systems may differ considerably, and again the localization and retention of the compound in specific brain structures cannot be excluded.

In conclusion, piribedil has an antiperoxidative effect in the brain in vivo, at least under oxidative stress, but not in vitro. This suggests that its in vivo action is related to the formation of an active metabolite, possibly the catechol derivative S584, which has antioxidant activity in both in vitro and in vivo models of oxidative stress. The mechanism(s) underlying this action are still not known, but might be related to the reported stimulatory effect of S584 on ATPase activity (Sawas and Gilbert, 1982) which would in turn depend on the presence of a catechol group in its structure.

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