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Differential neurobehavioral deficits induced by apomorphine and its oxidation product, 8-oxo-apomorphine-semiquinone, in rats

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Abstract

Apomorphine is a potent dopamine receptor agonist, which has been used in the therapy of Parkinson's disease. It has been proposed that apomorphine and other dopamine receptor agonists might induce neurotoxicity mediated by their quinone and semiquinone oxidation derivatives. The aim of the present study was to evaluate the possible neurobehavioral effects of apomorphine and its oxidation derivative, 8-oxo-apomorphine-semiquinone (8-OASQ). Adult female Wistar rats were treated with a systemic injection of apomorphine (0.05 or 0.5 mg/kg) or 8-OASQ (0.05 or 0.5 mg/kg) 20 min before behavioral testing. Apomorphine and 8-OASQ induced differential impairing effects on short- and long-term retention of an inhibitory avoidance task. Apomorphine, but not 8-OASQ, dose-dependently impaired habituation to a novel environment. The memory-impairing effects could not be attributed to reduced nociception or other nonspecific behavioral alterations, since neither apomorphine nor 8-OASQ affected footshock reactivity or behavior during exploration of an open field. The results suggest that oxidation products of dopamine or dopamine receptor agonists might induce cognitive deficits.

Keywords: Apomorphine; Dopamine; Oxidative stress; Parkinson's disease; Behavior; Memory

1. Introduction

The primary pathology characterizing Parkinson's disease is a selective degeneration of dopaminergic neurons in the substantia nigra, *pars compacta*, which project mainly to the striatum (Hirsch et al., 1988). It has been proposed that oxidative stress plays a pivotal role in the neurodegenerative damage associated with Parkinson's disease (Götz et al., 1990; Ben-Shachar et al., 1991; Fahn and Cohen, 1992; Youdim et al., 1994; Ebadi et al., 1996; Jenner, 1998). For instance, reduced glutathione levels (Sian et al., 1994), increased lipid peroxidation in substantia nigra (Dexter et al., 1994), and oxidative DNA damage (Spencer et al., 1994; Dragunow et al., 1997) have been suggested to be involved in the neurodegenerative mechanisms underlying Parkinson's disease.

The dopamine receptor agonist apomorphine has been used for the therapy of Parkinson's disease. Apomorphine is a potent dopamine D_1 and D_2 receptor agonists that promptly enters the brain and accumulates in the striatum (Bianchi and Landi, 1985). Although dopaminergic therapies have been suggested to enhance the progression of Parkinson's disease by producing reactive oxygen species and inducing cytotoxicity (Grandas and Obeso, 1989; Bindolli et al., 1992; Jenner and Brin, 1998; El-Bachá et al., 2001) and apomorphine at high concentration is toxic to cultured neurons (Spencer et al., 1994; Pardo et al., 1995; El-Bachá et al., 2001), there is no evidence for in vivo neurotoxicity of apomorphine. In fact, a growing body of evidence has shown a neuroprotective activity of apomorphine (Gassen et al., 1996, 1998; Grunblatt et al., 2001a,b). Apomorphine displays antiparkinsonian properties similar to those of L-DOPA and has been shown to be useful for treating Parkinson's disease patients, especially in the late

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stages of the disease (Di Chiara and Gessa, 1978; Corboy et al., 1995; Pzedborski et al., 1995).

Increasing evidence suggests that some of the effects of dopamine and dopamine receptor agonists might be mediated by their quinone and semiquinone oxidation derivatives (Graham, 1978; Bindolli et al., 1992; Smythies, 1997; Segura-Aguillar et al., 1998; El-Bachá et al., 2001). Dopamine oxidation derivatives such as dopamine *o*-quinone and *o*-semiquinone occur in the normal brain (Costa et al., 1992), and dopamine neurotoxicity has been suggested to be mediated by its quinone derivatives acting on NMDA glutamate receptors (see Smythies, 1997 for a review). Dopamine, apomorphine, and L-DOPA easily autoxidize, producing quinone and semiquinone derivatives that may lead to the formation of toxic products and superoxide radicals (Graham, 1978; Bindolli et al., 1992; Segura-Aguillar et al., 1998; El-Bachá et al., 2001), and the toxic effects of apomorphine to cultured neurons have been shown to correlate to its autoxidation (El-Bachá et al., 2001). Recently, we described the isolation of an apomorphine autoxidation semiquinone derivative, 8-oxo-apomorphine-semiquinone (8-OASQ), and demonstrated for the first time its mutagenic activity in vitro (Khromov-Borisov et al., 2000).

Given the neuroprotective properties and clinical relevance of apomorphine in the therapy of Parkinson's disease, and the possibility that autoxidation derivatives of apomorphine are involved in mediating its neurobiological effects, it is important to investigate the neurobehavioral and possible neurotoxic properties of apomorphine and its oxidation derivatives in vivo. In the present study, we evaluated the effects of the systemic administration of apomorphine and its autoxidation derivative, 8-OASQ, on aversive memory, habituation, and open field behavior in rats.

2. Materials and methods

2.1. Animals

We have obtained 182 adult female Wistar rats (170–320 g) from our breeding colony. They were housed five to a cage with food and water available ad libitum, and were maintained on a 12-h light/dark cycle (lights on at 07:00 h). All behavioral procedures were conducted between 10:00 and 16:00 h. All experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care.

2.2. Drugs and pharmacological procedures

Apomorphine HCl (Sigma) was dissolved in saline with 10% dimethyl sulfoxide (DMSO). 8-OASQ was isolated as a black water-insoluble precipitate after the incubation of apomorphine HCl (5 mg/ml) for 2 days as previously described (Khromov-Borisov et al., 2000). Isolated 8-OASQ was then dissolved in saline with 10% DMSO. Twenty minutes prior to the behavioral procedures, animals were given an intraperitoneal (i.p.) injection of vehicle (10% DMSO in saline), apomorphine (0.05 or 0.5 mg/kg), or 8-OASQ (0.05 or 0.5 mg/kg), in a volume of 1.0 ml/kg body weight. The doses were chosen on the basis of previous reports on the behavioral effects of apomorphine in rats (Doyle and Regan, 1993; Doyle et al., 1996). All solutions were prepared immediately before injections.

2.3. Behavioral procedures

2.3.1. Inhibitory avoidance

Inhibitory avoidance in rodents is a widely used animal model of aversive learning and memory. The step-down inhibitory avoidance apparatus and procedures were described in previous reports (Izquierdo et al., 1997; Roesler et al., 1999, 2000). The inhibitory avoidance box was a $50 \times 25 \times 25$ cm acrylic box whose floor consisted of parallel stainless steel bars (1 mm diameter) spaced 1 cm apart. A 7-cm-wide, 2.5-cm-high platform was placed on the floor of the box against the left wall. Animals were placed on the platform and their latency to step-down on the grid with all four paws was recorded with an automated device. In training sessions, immediately after stepping down on the grid, the animals were given a 0.6-mA, 1.0-s footshock. In retention test sessions, carried out 1.5 h (short-term retention) or 48 h (long-term retention) after training, no footshock was given and the step-down latency (maximum 180 s) was used as a measure of retention.

2.3.2. Footshock reactivity

The footshock sensitivity test was carried out in the same apparatus used for inhibitory avoidance, as described in previous reports (Roesler et al., 1999, 2000). A modified version of the "up-and-down" method (Crocker and Russell, 1984) was used to determine the nociceptive thresholds. The platform was removed and each animal was placed on the grid and allowed a 1-min habituation period prior to the start of a series of footshocks (0.5 s) delivered at 10-s intervals. Shock intensities ranged from 0.1 to 0.6 mA in 0.1-mA increments. The adjustments in shock intensity were made in accordance to each animal's response. Shock intensity was raised by 1 unit when no response occurred and lowered by 1 unit when a response was made. A "flinch" response was defined as withdrawal of one paw from the grid floor, and a "jump" response was defined as a rapid withdrawal of three or four paws. Two measurements of the "flinch" threshold were made and then two measures of the "jump" threshold were made. For each animal, the mean of the two scores for the flinch and jump thresholds was calculated.

2.3.3. Open field behavior

Animals used in the open field and habituation experiments had been previously trained in inhibitory avoidance.

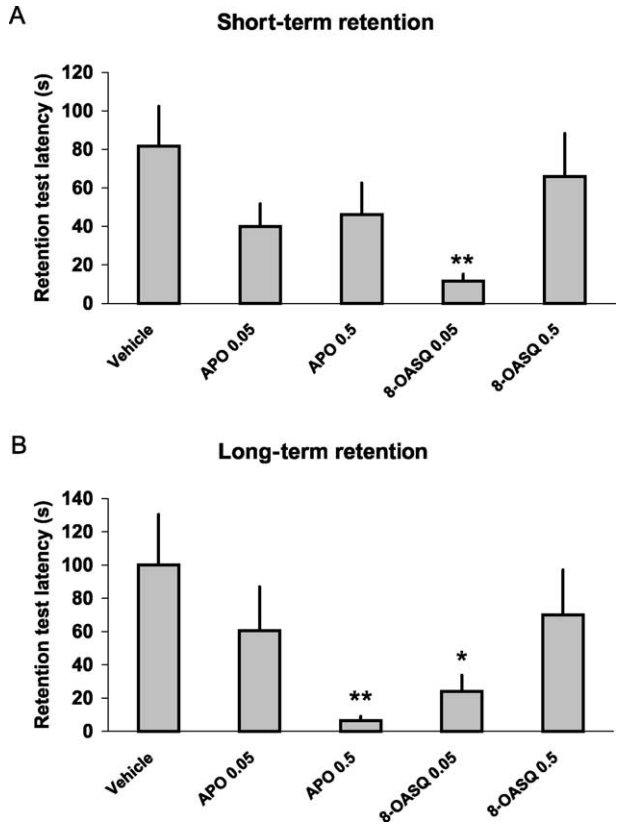

Fig. 1. Effect of pretraining administration of apomorphine (APO) (0.05 or 0.5 mg/kg) and 8-oxo-apomorphine-semiquinone (8-OASQ) (0.05 or 0.5 mg/kg) on (A) short- (1.5 h after training) and (B) long-term (48 h after training) retention of inhibitory avoidance. Animals were given an i.p. injection of vehicle, apomorphine, or 8-OASQ 20 min prior to training. Data are means ± S.E. retention test latencies (s). $N = 8-10$ animals per group; * $P < 0.05$ and ** $P < 0.01$ compared to the vehicle group.

The open field was 50 × 25 cm, surrounded by 50-cm high walls, made of brown plywood with a frontal glass wall. The floor of the open field was divided into 12 equal squares by black lines. One week after the end of the inhibitory avoidance experiment, rats were put in the open field, placed on its left rear quadrant, and left to freely explore the arena for 5 min. Crossing of the black lines, rearings performed, latency to start locomotion, and the number of fecal pellets produced during exploration were counted and used as measures of locomotion, exploration, motivation, and anxiety (Roesler et al., 2000).

2.3.4. Habituation

Long-term retention of habituation to a novel environment can be considered a nonassociative, nonaversive type of learning, which can be measured by the decrease in the exploratory activity as assessed by the number of rearings performed in a test session carried out 24 h after the first exploration session (Vianna et al., 2000). The animals used in the evaluation of open field behavior were reexposed (test session) for 5 min to the open field 24 h after the first exposure (training session), and the number of rearings performed was recorded. The decrease in the number of rearings performed between the first and the second exploration sessions was taken as a measure of habituation.

2.4. Statistics

Data are expressed as mean ± S.E. Differences among groups were analysed with a one-way analysis of variance (ANOVA) followed by an LSD post-hoc test when necessary. Comparisons between the number of rearings performed in training and test sessions within the same group in the habituation experiment were done with a paired *t*-test. In all comparisons, $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Effects of pretraining administration of apomorphine or 8-OASQ on short- and long-term retention of inhibitory avoidance

Short- and long-term retention of inhibitory avoidance were evaluated in different animals. There were no significant differences among groups in training performance (short-term retention experiment, $P = 0.44$; overall mean ± S.E. training latency = 12.83 ± 1.47; long-term retention experiment; $P = 2.47$; overall mean training latency = 8.59 ± 1.18). Fig. 1A shows the short-term (1.5 h) retention of inhibitory avoidance in rats given an i.p. injection of vehicle, apomorphine (0.05 or 0.5 mg/kg), or 8-OASQ (0.05 or 0.5 mg/kg) 20 min prior to training. 8-OASQ at the dose of 0.05 mg/kg, but not apomorphine at either dose used or 8-

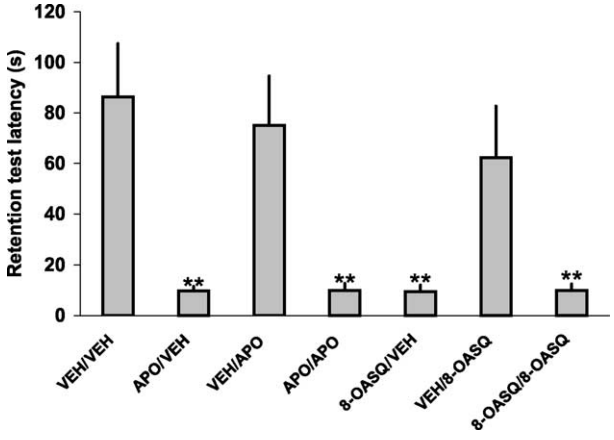

Fig. 2. Effect of combined pretraining and pretest administration of apomorphine (APO) (0.5 mg/kg) and 8-oxo-apomorphine-semiquinone (8-OASQ) (0.05 mg/kg) on long-term (48 h after training) retention of inhibitory avoidance. Animals were given an i.p. injection of vehicle (VEH), apomorphine, or 8-OASQ 20 min prior to training and 20 min prior to test. Data are means ± S.E. retention test latencies (s). $N = 10$ animals per group; ** $P < 0.01$ compared to the VEH/VEH group.

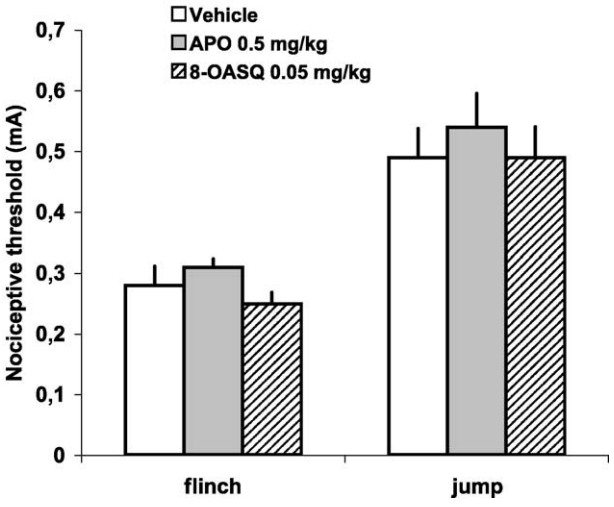

Fig. 3. Effect of apomorphine (APO) (0.5 mg/kg) and 8-oxo-apomorphine-semiquinone (8-OASQ) (0.05 mg/kg) on reactivity to the footshock. Animals were given an i.p. injection of vehicle, apomorphine, or 8-OASQ 20 min prior to testing nociceptive thresholds. Data are means ± S.E. nociceptive thresholds (mA). $N = 7$ animals per group. There were no significant differences between groups.

OASQ at the dose of 0.5 mg/kg, impaired short-term inhibitory avoidance retention. The effects of apomorphine and 8-OASQ on long-term (48 h) retention is shown in Fig. 1B. Apomorphine at the dose of 0.5 mg/kg and 8-OASQ at the dose of 0.05 mg/kg, but not apomorphine at the dose of 0.05 mg/kg or 8-OASQ at the dose of 0.5 mg/kg, impaired retention test performance. When retrained and retested drug-free 1 week after the first training, animals previously treated with 0.5 mg/kg apomorphine or 0.05 mg/kg 8-OASQ and tested for 48-h retention showed normal inhibitory avoidance learning ability (mean ± S.E. overall drug-free training latency = 14.71 ± 1.97, $P = 0.93$; mean ± S.E. 48-h retention test latency = 159.53 ± 20.48 in the animals previously treated with apomorphine 0.5 mg/kg, and 139.85 ± 26.30 in animals previously treated with 8-OASQ 0.05 mg/kg), indicating that the impairing effects of apomorphine and 8-OASQ could not be attributed to an irreversible impairing effect or permanent neuronal damage.

3.2. Effects of combined pretraining and pretest administration of apomorphine and 8-OASQ on long-term retention of inhibitory avoidance

In order to evaluate the possible contribution of state dependency on the memory-impairing effects of apomorphine and 8-OASQ, as well as the possible effects of apomorphine and 8-OASQ on memory retrieval, we verified the effects of combined pretraining and pretest injections of memory-impairing doses of apomorphine and 8-OASQ on inhibitory avoidance retention. Thus, the resulting pretraining/pretest treatment groups were vehicle/vehicle, apomorphine/vehicle, vehicle/apomorphine, apomorphine/apmorphine, 8-OASQ/vehicle, vehicle/8-OASQ, and 8-

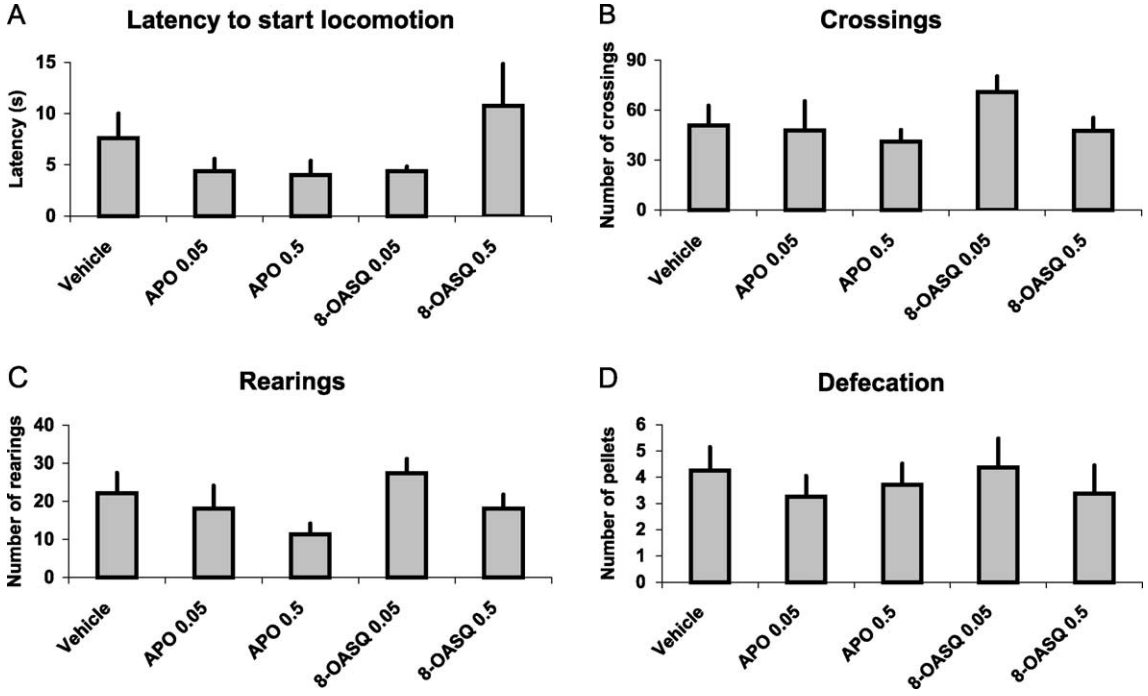

Fig. 4. Effect of pretraining administration of apomorphine (APO) (0.05 or 0.5 mg/kg) and 8-oxo-apomorphine-semiquinone (8-OASQ) (0.05 or 0.5 mg/kg) on the (A) latency to start locomotion, (B) number of crossings performed, (C) number of rearings performed, and (D) number of fecal pellets produced during a 5-min exploration of an open field. Animals received an i.p. injection of vehicle, apomorphine, or 8-OASQ 20 min prior to being exposed to the open field. Data are expressed as means ± S.E. $N = 7–8$ animals per group. There were no significant differences between groups.

OASQ/8-OASQ. There were no significant differences among groups in training performance ($P = 0.89$; overall mean ± S.E. training latency = 10.01 ± 0.70). Retention test latencies are shown in Fig. 2. Combined pretraining and pretest treatments with either apomorphine or 8-OASQ failed to reverse the retention deficits, indicating that the impairing effects do not involve state dependency. Moreover, pretest administration of apomorphine or 8-OASQ did not affect retrieval, as shown by the normal retention in the vehicle/apomorphine and vehicle/8-OASQ groups, showing that those drugs impaired the formation, but not the expression of inhibitory avoidance memory.

3.3. Lack of effect of apomorphine and 8-OASQ on reactivity to the footshock

In order to verify whether the impairing effects of apomorphine and 8-OASQ on memory for inhibitory avoidance could be due to a reduction in nociception, we evaluated the effects of apomorphine and 8-OASQ at the doses shown to be effective in inhibitory avoidance on footshock sensitivity. Reactivity to the footshock assessed by flinch and jump thresholds was not affected by apomorphine or 8-OASQ (Fig. 3), indicating that the memory-impairing effects of pretraining injections of those drugs were not due to reduced nociceptive response.

3.4. Lack of effect of apomorphine and 8-OASQ on open field behavior

Behavior during a 5-min exploration of an open field in rats given apomorphine or 8-OASQ 20 min pretraining is shown in Fig. 4. One animal treated with apomorphine 0.5 mg/kg showed stereotyped behavior (rapid, repetitive forelimb and head movements) during open field exploration and was excluded from the experiment. There were no significant differences among groups in the number of crossings or rearings performed, latency to start locomotion, or number of fecal pellets produced. These findings suggest that apomorphine and 8-OASQ did not affect locomotion, exploration, motivation, or anxiety.

3.5. Effects of apomorphine and 8-OASQ on habituation to an open field

The habituation of rearings in rats submitted to a training and a test session of exploration in the open field is shown in Fig. 5. The group treated with 0.5 mg/kg of apomorphine 20 min pretraining showed a significantly higher number of rearings during test compared to the vehicle-treated group. In addition, all groups showed a significant decrease in the number of rearings in the test session in comparison with the training session, except the group given apomorphine at 0.5 mg/kg (comparisons between training and test sessions within each group by paired *t*-tests, $P = 0.27$ in the apomorphine 0.5 mg/kg-treated group). These results show that apomorphine, but not 8-OASQ, dose-dependently impaired long-term habituation to a novel environment.

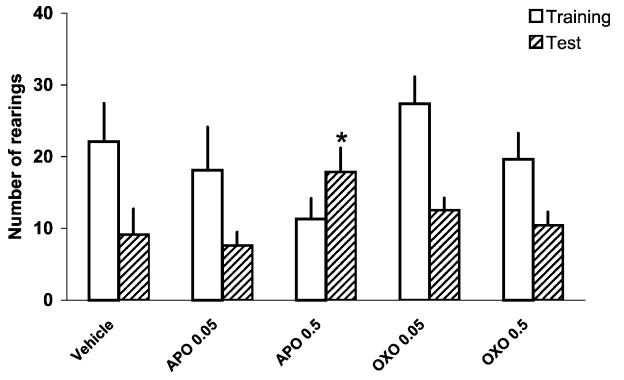

Fig. 5. Effect of pretraining administration of apomorphine (APO) (0.05 or 0.5 mg/kg) and 8-oxo-apomorphine-semiquinone (8-OASQ) (0.05 or 0.5 mg/kg) on habituation to an open field. Animals received an i.p. injection of vehicle, apomorphine, or 8-OASQ 20 min prior to training. Data are means ± S.E. number of rearings performed. $N = 7–8$ animals per group; * $P < 0.05$ compared to the vehicle group.

4. Discussion

The results of the present study show that systemic pretraining administration of the dopamine receptor agonist apomorphine and the oxidation product of apomorphine, 8-OASQ, induced differential impairing effects on short- and long-term retention of a step-down inhibitory avoidance task in rats, whereas apomorphine, but not 8-OASQ, impaired habituation. The impairing effects of apomorphine and 8-OASQ on inhibitory avoidance were not due to state dependency. Neither drug affected inhibitory avoidance retrieval, footshock reactivity, or behavior during exploration of an open field.

Apomorphine is a classical nonselective dopamine receptor agonist and antiparkinsonian therapeutic agent (Di Chiara and Gessa, 1978; Bianchi and Landi, 1985; Corboy et al., 1995; Pzedborski et al., 1995). Although the main clinical symptoms of Parkinson's disease are motor disturbances (Duvoisin, 1991), animal studies have suggested that Parkinson's disease also involves learning and memory disabilities (Roeltgen and Schneider, 1994; Fernandez Ruiz et al., 1995; Brown et al., 1997; Da Cunha et al., 2001), and Parkinson's disease patients show cognitive deficits, including memory impairments (Dubois and Pillon, 1997; Goldman et al., 1998). Thus, it is important to characterize the cognitive effects of dopaminergic drugs used in Parkinson's disease therapy and their metabolites. Some of the findings described in the present report, namely the impairing effect of apomorphine on inhibitory avoidance and the lack of effect on open field exploration, are consistent with previous studies (Davies et al., 1974; Ichihara et al., 1988; Doyle and Regan, 1993; Doyle et al., 1996). The aversive memory-impairing effect of apomorphine reported in the present

study suggests that activation of central dopaminergic receptors by apomorphine dose-dependently impair the formation, but not the expression of long-term inhibitory avoidance memory, and retention of the nonaversive, non-associative habituation task, without affecting short-term retention, locomotion, motivation, anxiety, or nociception. By contrast, the oxidation derivative of apomorphine, 8-OASQ, at the lower but not at the higher dose used, impaired both short- and long-term retention of inhibitory avoidance without affecting habituation, suggesting that apomorphine and its oxidized product 8-OASQ display differential pharmacological actions. Although several drugs have been shown to affect memory in lower doses while higher doses are ineffective, further studies are necessary to clarify the dose–response pattern of the mnemonic effect of 8-OASQ and the mechanisms underlying it.

Although in vitro studies show that apomorphine can be toxic to cultured neurons, inducing cytotoxicity and DNA damage through oxidative stress (Spencer et al., 1994; Pardo et al., 1995; El-Bachá et al., 2001), evidence for in vivo neurotoxicity of apomorphine remains lacking, and apomorphine has been proposed as a potential neuroprotective in vivo (Gassen et al., 1998; Chen et al., 2001; Fornai et al., 2001). Increasing evidence suggests that apomorphine displays a neuroprotective activity mediated by its free radical scavenging properties (Mytilineou et al., 1993; Gassen et al., 1996, 1998; Chen et al., 2001; Grunblatt et al., 2001a,b). Both *R*-apomorphine and its isomer, *S*-apomorphine, which is not a dopamine receptor agonist, have been shown to exert neuroprotective effects in the *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mice model of Parkinson's disease (Grunblatt et al., 2001a).

Recent studies suggest that some of the neurobiological effects of dopamine and dopamine receptor agonists such as apomorphine might be mediated by their oxidation products, and the effects of dopamine receptor agonists quinone and semiquinone derivatives might be importantly involved in the neurodegeneration associated with Parkinson's disease (Graham, 1978; Bindolli et al., 1992; Smythies, 1997; Segura-Aguillar et al., 1998; El-Bachá et al., 2001). Although the mechanisms underlying the effects of dopamine receptor agonists quinone and semiquinone derivatives are still unclear, there is evidence indicating that they stimulate NMDA receptor-mediated excitotoxicity by acting on NMDA receptors (Smythies, 1997), bind irreversibly to intracellular proteins forming conjugates (El-Bachá et al., 2001), and induce the formation of reactive oxygen species, initiating intracellular oxidative stress (Graham, 1978; Bindolli et al., 1992; Segura-Aguillar et al., 1998; El-Bachá et al., 2001).

To our knowledge, the present study is the first to evaluate the neurobehavioral activity of an isolated apomorphine autoxidation derivative. Our findings show that a single, systemic administration of 8-OASQ, at doses comparable to memory-impairing doses of apomorphine, is capable of inducing impairments on retention of an aversive memory task without affecting other behavioral parameters. The effects were not due to a long-lasting impairing effect or permanent neuronal damage since rats treated with either apomorphine or 8-OASQ were able to learn normally when retrained drug-free 1 week later. Moreover, 8-OASQ affected memory at the lowest, but not at the highest dose used.

The present findings suggest that oxidation derivatives of apomorphine and possibly those of dopamine and L-DOPA clinically used in the therapy of Parkinson's disease might display pharmacological actions on mechanisms involved in emotional learning and memory processes and induce cognitive deficits. An interesting possibility is that 8-OASQ might have antioxidant properties and thus exert neuroprotective effects. Further experiments are currently being carried out in our laboratory in order to investigate this possibility, as well as to clarify the cellular and molecular mechanisms underlying the neurobehavioral effects of 8-OASQ.

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