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Differences in pre- and post-synaptic sensitivity to apomorphine between saline and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated C57BL/6 mice as reflected in climbing activity

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Abstract

The climbing behaviour after low doses (0.05, 0.1 and 0.2 mg/kg) or a high dose (1.5 mg/kg) of apomorphine was studied in saline or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated C57BL/6 mice. Following a 3-week recovery from two injections of saline or MPTP (50 mg/kg inter-injection period: 72 h), mice were randomly selected for determinations of contents of neurotransmitters and metabolites (dopamine, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-hydroxytryptamine, 5-HT) and 5-hydroxyindole-3-acetic acid (5-HIAA)) or the apomorphine-induced climbing paradigm. For the climbing experiment, the animals were habituated for 60 min to metal climbing cylinders after which they received a subcutaneous injection of apomorphine or its solvent. Subsequently, the animals were placed back in the cylinders and their climbing scores were recorded every 5 min for 60 min. The biochemical data indicated that striatal levels of dopamine, DOPAC and HVA were significantly reduced following MPTP-treatment whereas striatal 5-HT and 5-HIAA levels were unaffected. In the climbing paradigm saline and MPTP-treated C57BL/6 mice responded diametrically opposite to low doses of apomorphine: 0.1 and 0.2 mg/kg apomorphine reduced the climbing score in saline-treated mice as compared to saline injections whereas 0.2 mg/kg apomorphine increased the climbing score in MPTP-treated mice. A relatively high dose of apomorphine (1.5 mg/kg) increased the climbing score in both saline- and MPTP-treated mice. However, the climbing score was significantly higher in MPTP-treated mice than in saline-treated mice. These data suggest that MPTP-treated mice lack pre-synaptic dopamine receptors and have an increased post-synaptic sensitivity for apomorphine which is in agreement with the fact that MPTP selectively affects the dopaminergic nigro-striatal pathway which then results in an up-regulation of post-synaptic receptors.

Keywords: MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine); Apomorphine; Climbing; Dopamine; (Mouse); C57BL/6

1. Introduction

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) selectively destroys dopaminergic cells in the substantia nigra of C57BL/6 mice, primates and man (Davis et al., 1978; Langston et al., 1983; Burns et al., 1983; Heikkila et al., 1984). For this particular characteristic MPTP has been extensively used in animal models for Parkinson's disease. In contrast to primates and man in which treatment with MPTP results in conspicuous symptoms known to occur in Parkinson's disease (Davis et al., 1978; Langston et al., 1983; Burns et al., 1983), rodents display relatively limited behavioural changes following MPTP treatment despite a robust depletion of striatal dopamine levels (Willis and Donnan, 1987, Sundström et al., 1990; Sundström et al., 1994). Furthermore, equivocal results have been described with different stocks of animals using comparable procedures [for review, see the work of Sundström et al. (1994)]. These findings have hampered the development of a simple rodent analog of the MPTP primate model.

Nevertheless, in pharmacological studies MPTP has been widely used in rodents to study receptor plasticity (Lau and Fung, 1986; Wiener et al., 1989; Fredriksson et al., 1990; Weihmuller et al., 1990). However, regarding pharmaco-behavioural indices of receptor alterations equivocal results have been reported from different laboratories even when using comparable tests such as the apomorphine-induced climbing. Whereas Sundström et al. (1990) found no difference in the apomorphine-induced

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climbing activity between saline and MPTP-treated C57BL/6 mice, in the study by Lau and Fung (1986) saline and MPTP-treated CF1 mice behaved different. Interestingly, apart from using different mouse strains, another basic difference between the two studies is the habituation to the test situation. Lau and Fung (1986) habituated their mice for 30 min to the climbing cages whereas in the study of Sundström et al. (1990) the animals were immediately tested in the climbing paradigm. Habituation or pre-exposure to the test situation is known to alter the behavioural response of animals to drugs (cf. Gendreau et al., 1997; Starr and Starr, 1995).

Given the above-mentioned discrepancy we decided to study the effects of apomorphine in the climbing paradigm in saline- or MPTP-pre-treated C57BL/6 mice using extended habituation (60 min) prior to testing. Furthermore, we included both low (sub-threshold) doses (0.05, 0.1 and 0.2 mg/kg) and a high dose of apomorphine (stimulating post-synaptic receptors; 1.5 mg/kg).

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (strain: C57BL/6JTif (SPF); $n = 240$; 22–25 g) were individually housed in macrolon cages ($21 \times 34 \times 14$ cm) in a temperature-controlled room under artificial illumination (0600 h–1800 h, lights on) with access to water and food (Ecosan, Eberle Nafag AG, Gossau, Schweiz), ad libitum.

2.2. MPTP injections

The animals received two subcutaneous injections (behind the neck; volume 10 ml/kg) of either MPTP–HCl (50 mg/kg, RBI; $n = 120$) or saline ($n = 90$) with an inter-injection interval of 72 h. Subsequent to each injection the animals were placed back in their home-cage. Following the second injection the animals were allowed to recover for at least 21 days before testing.

2.3. Apomorphine-induced climbing procedures

The apomorphine-induced climbing paradigm was adopted from the work of Protais et al. (1976) with minor modifications. Briefly, each animal was individually habituated to a cylindrical cage (diameter 12 cm, 14-cm high, with walls of vertical metal bars, 2-mm diameter, 1-cm apart, mounted on a smooth surface) for 60 min. Subsequently the animals were randomly selected to receive a subcutaneous injection (into the neck) of saline or apomorphine (doses 0.05, 0.1, 0.2 or 1.5 mg/kg) and placed back in the cylinders for an additional 60 min. During this period the 'apomorphine-induced climbing activity' was manually recorded every 5 min using the following score: 0: four paws on the floor; 1: forepaw(s) holding (a) bar(s); 2: four paws holding bars. Cumulated scores over 60 min were used to quantify the behavioural response of each mouse. The number of animals per experimental group was 15–18.

2.4. Brain tissue dissection

An additional group of animals was injected according to the above-mentioned injection schedule ($n = 15$ per group (saline and MPTP)) and subsequently used for neurochemical determinations. Four weeks after the final MPTP injection the animals were decapitated and their brains were quickly removed on ice. The left and right striata were dissected and stored in centrifuge tubes at -20°C.

2.5. HPLC determinations

Striatal dopamine (DA, dopamine), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), sero-

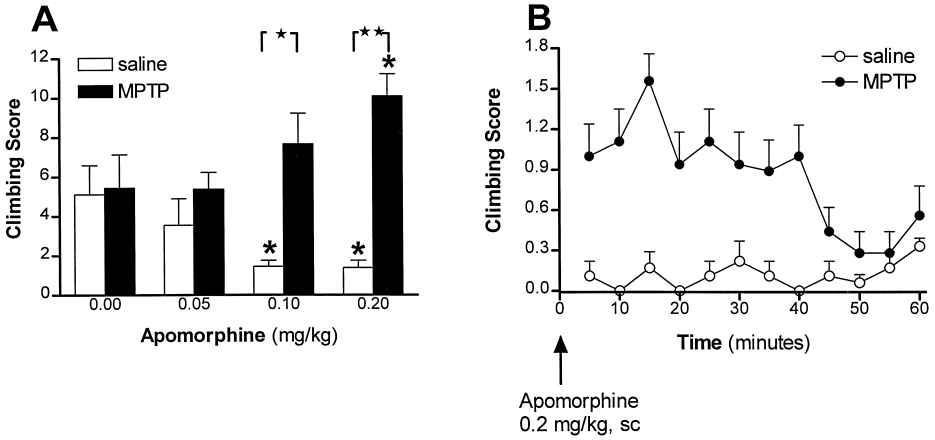

Fig. 1. Effects of low doses of apomorphine on climbing. (A) Bars represent the mean climbing scores (±SEM) per 60 min per dose of apomorphine. (B) Time course of climbing scores per 5 min (±SEM) following the injection of 0.2 mg/kg apomorphine; statistics (for details see Section 2: $* = p < 0.05$ vs. saline (0 mg/kg); $★ = p < 0.0004$/$★★ = p < 0.0001$.

tonin (5-hydroxytryptamine, 5-HT) and 5-hydroxyindole-3-acetic acid (5-HIAA) concentrations were determined by means of HPLC. Striata were sonicated in 2 ml of the mobile phase for the HPLC separations described below, containing 100 ng α-methyl-DOPA per extract as an internal standard. Cell debris were removed by centrifugation. A total of 200 μl of the supernatant were automatically injected (Waters 717 Autosampler, Waters Ass., Milford, USA) into a liquid chromatography system (Bioanalytical Systems, W. Lafayette, USA) fitted with a C_{18}-μBondapak reversed phase column (Waters Ass.) and a LC4B Amperometric detector (Bioanalytics Systems). The oxidation potential was set at +0.7 V. The mobile phase contained chloracetic acid (0.15 M), acetonitrile (8%), sodiumoctylsulfate (650 μM) and EDTA (0.7 mM) and was adjusted to pH 3.0 with NaOH. The column temperature was set at 28°C and a flow rate of 0.8 ml/min was used. With these parameters optimal separation was achieved with a minimal detection level of 20 nA/V.

2.6. Statistics

The biochemical values were normalized and statistically evaluated using a (one-way) analysis of variance (ANOVA) followed by a pairwise post-hoc Student's *t*-test (two-tailed). The climbing scores of the two treatment groups to the different doses of apomorphine were evaluated by means of a two (factors: treatment × dose) ANOVA followed by a post-hoc Dunnett's test (two-tailed; all compared to control (0 mg/kg)) or Student's *t*-test (two-tailed), where appropriate. Differences were considered significant when $p < 0.05$.

3. Results

3.1. Climbing activity

3.1.1. Effects of low doses of apomorphine (0.05, 0.1 and 0.2 mg / kg)

The ANOVA indicated a statistically significant interaction between treatment and dose ($p < 0.002$). The post-hoc analysis revealed that saline-treated mice had reduced climbing scores following 0.1 and 0.2 but not 0.05 mg/kg apomorphine as compared to solvent (Fig. 1A). MPTP-treated mice had an increased climbing score at dose 0.2 mg/kg but not at doses 0.05 and 0.1 mg/kg apomorphine as compared to solvent (Fig. 1A).

The ANOVA indicated a highly statistically significant effect for factor treatment (ANOVA, $p < 0.001$). Post-hoc analysis revealed that the climbing scores of saline- and MPTP-treated C57BL/6 mice differed significantly at doses 0.1 and 0.2 mg/kg apomorphine (Fig. 1A). The climbing scores following saline and 0.05 mg/kg apomorphine did not significantly differ between saline- and MPTP-treated mice.

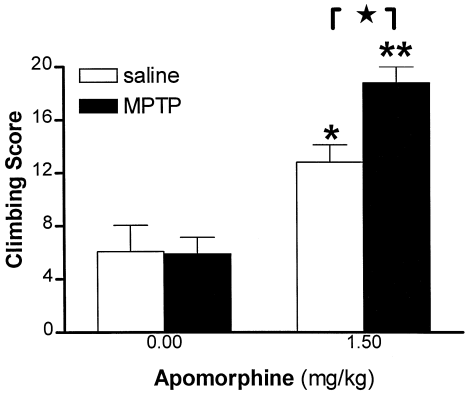

Fig. 2. Effects of a high dose of apomorphine on climbing. Bars represent the mean climbing scores (±SEM) per 60 min per dose of apomorphine (0 and 1.5 mg/kg). Statistics (for details see Section 2): * = $p < 0.005$/** = $p < 0.0001$ vs. saline (0 mg/kg); ★ = $p < 0.005$.

3.1.2. Effects of a high dose of apomorphine (1.5 mg / kg)

The ANOVA indicated a statistically significant interaction between treatment and dose ($p < 0.04$). Post-hoc analysis revealed that the climbing scores of saline- and MPTP-treated C57BL/6 mice were significantly increased in response to 1.5 mg/kg apomorphine (Fig. 2). However, the climbing response of MPTP-treated mice was significantly higher than the climbing response of saline-treated mice (Fig. 2).

3.2. Biochemical determinations

Comparing saline and MPTP-treated mice, a highly significant treatment effect was found (ANOVA, $p < 0.0001$). Post-hoc analysis revealed that striatal DA, DOPAC and HVA concentrations were significantly reduced following MPTP treatment by 96% (434 ± 68 ng/g), 92% (397 ± 34 ng/g) and 67% (611 ± 39 ng/g), respectively as compared to control values (DA: 11335 ± 391 ng/g; DOPAC: 4869 ± 313 ng/kg; HVA: 1859 ± 92

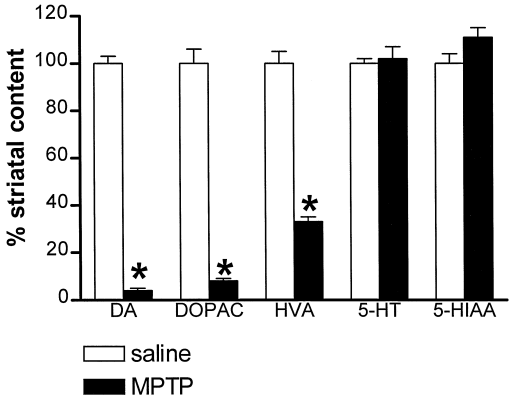

Fig. 3. Relative striatal contents of DA, DOPAC, HVA, serotonin (5-HT) and 5-HIAA 4 weeks following subcutaneous injections of saline or MPTP (2×50 mg/kg, s.c., 72-h inter-injection period). Statistics: ANOVA ($p < 0.0001$), Student's *t*-test, * = $p < 0.0001$ vs. saline.

ng/g; Fig. 3). In contrast, MPTP did not significantly alter striatal 5-HT (102%: 1138 ± 56 ng/g) or 5-HIAA (111%: 777 ± 27 ng/g) concentrations as compared to control values (5-HT: 1112 ± 26 ng/g; 5-HIAA: 698 ± 27 ng/g; Fig. 3).

4. Discussion

The present data clearly demonstrate that saline- and MPTP-treated C57BL/6 mice respond diametrically opposite to low doses of apomorphine when using apomorphine-induced climbing. The reduced climbing scores observed in the saline-treated C57BL/6 mice reflect pre-synaptic receptor stimulation that occurs at these low doses of apomorphine. The absence of this response in MPTP-treated mice then indicates that these receptors are located on the dopaminergic terminals which are virtually completely destroyed following treatment with MPTP (Heikkila et al., 1984). A physiological consequence of such a dopaminergic denervation is then an up-regulation of (post-synaptic) dopamine receptors which may explain the increased climbing scores of the MPTP-treated mice at a dose as low as 0.2 mg/kg apomorphine, i.e., a dose with normally minor/no (post-synaptic) activity in this test (Protais et al., 1976; Vasse and Protais, 1989). Accordingly, by using this dose of apomorphine (0.2 mg/kg) saline-treated mice and MPTP-treated mice are clearly distinct in the apomorphine-induced climbing paradigm.

The increased climbing scores in both treatment groups following the high dose of apomorphine (1.5 mg/kg) is in line with the stimulation of post-synaptic receptors (Protais et al., 1976). Here, the climbing scores of MPTP-treated mice was higher than that of the saline-treated mice which probably reflects the sensitization of the dopamine receptor system following denervation with MPTP (Lau and Fung, 1986; Wiener et al., 1989; Weihmuller et al., 1990).

When comparing the present findings with those of Sundström et al. (1990), the difference between the two studies is probably due to the fact that apomorphine was given immediately prior to testing in the latter study whereas the data obtained here were collected in previously habituated animals. Other (laboratory specific) experimental conditions may obviously also play a role. One of such factors clearly is the level of dopamine depletion of the striatum. However, in the study of Lau and Fung (1986) striatal dopamine levels were reduced by 71% whereas Sundström et al. (1990) achieved a depletion level of 80%. In the present experiment dopamine levels were reduced by 96%. These data indicate that different levels of dopamine depletion cannot account for, or contribute in the differential findings of these studies.

On the basis of our data we conclude that both the pre-synaptic and post-synaptic sensitivity to apomorphine in saline- and MPTP-treated mice can be differentiated using the apomorphine-induced climbing activity and that the initially high level of exploration may mask relevant differences between saline- and MPTP-treated C57BL/6 mice in the climbing paradigm.

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