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Effects of adenosine A_1, dopamine D1 and metabotropic glutamate 5 receptors-modulating agents on locomotion of the reserpinised rats

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Abstract

The pathophysiology of Parkinson's disease and L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia are characterised by an imbalance between activity of the direct and indirect pathways regulated by dopamine D1 and D2 receptors, respectively. In this study, we investigated the effects of treatments combining adenosine A_1 and metabotropic glutamate 5 (mGlu5) receptors modulators on locomotion induced by dopamine D1 receptor activation in the reserpine-treated rats. Administration of the adenosine A_1 receptor agonist and mGlu5 receptor antagonist resulted in the significant reduction of dopamine D1 receptor agonist-induced locomotion. The combination of adenosine A_1 receptor agonist with mGlu5 receptor antagonist had no greater effect than these compounds alone. However, the adenosine A_1 receptor antagonist attenuated the inhibitory effect of mGlu5 receptor antagonist. The data suggest that the effect of mGlu5 receptor blockade on locomotion elicited by dopamine D1 receptor stimulation involves activation of adenosine A_1 receptors. This interaction can improve our understanding of pathophysiology of L-DOPA-induced dyskinesia.

Keywords: mGlu5 receptor; Adenosine receptor; Dopamine receptor; Parkinson's disease; L-DOPA-induced dyskinesia; Locomotion

1. Introduction

Parkinson's disease is a neurodegenerative movement disorder characterised by reduced locomotion due to depletion of dopamine in the striatum. The pathophysiological mechanism underlying the parkinsonian state involve a distortion of the levels of activity between the striatonigral (direct pathway) and striatopallidal projections (indirect pathway), which are regulated by dopamine D1 and D2 receptors, respectively (Alexander, 1994; Crossman, 2000; Kingsley, 2000). The most common treatment of Parkinson's disease is based upon dopamine replacement by L-3,4-dihydroxyphenylalanine, L-DOPA, a dopamine precursor (Feldman et al., 1997). However, long-term treatment with L-DOPA causes disabling complications, in particular dyskinesia, characterised by involuntary movements. It has been proposed that a key pathophysiological component of L-DOPA-induced dyskinesia is overactivity in the striatonigral projection pathway (Bezard et al., 2001; Brotchie, 1998).

The loss of more than 90% of striatal dopamine levels leads to an imbalance in reciprocal interactions between glutamatergic and dopaminergic transmission in the basal ganglia (Blandini et al., 2000; Lange et al., 1997; Starr, 1995). Enhanced glutamate transmission in regions of the motor loop is likely mediated through various glutamate receptors (Marino et al., 2003; Nash et al., 1999; Ossowska, 1994; Ossowska et al., 2002). Metabotropic glutamate 5 receptor (mGlu5 receptor) is abundantly expressed in the basal ganglia, particularly in the striatum (Romano et al., 1995; Tallaksen-Greene et al., 1998; Testa et al., 1995) and is also involved in controlling activity of striatal medium spiny neurons (Diaz-Cabiale et al., 2002; Mao and Wang,

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2001; Parelkar and Wang, 2003). However, the potential role of mGlu5 receptor in L-DOPA-induced dyskinesia, or in regulating other behaviours involving dopamine D1 receptor signalling, has not been investigated in detail.

Dopaminergic functions are also modulated by adenosine (Ferre et al., 1992, 1997, 1998; Okada et al., 1996; Popoli et al., 1994; Rimondini et al., 1998) and adenosine–dopamine receptor interactions may be responsible for regulations of locomotor activity. For instance, the stimulation of adenosine A_1 receptors has a powerful inhibitory effect on dopamine D1 receptors (Ferre et al., 1994, 1996, 1999; Popoli et al., 1996a), whereas the blockade of adenosine A_1 receptor potentiates motor activity elicited by dopamine D1 receptors (Popoli et al., 1996b). Adenosine A_1 receptors have been identified on the same γ-aminobutyric acid (GABA)ergic neuronal cells as dopamine D1 receptors, the direct pathway (Fuxe et al., 1998). Therefore, it can be suggested that modulation of the former may potentially affect locomotion induced by the activation of the direct pathway.

Given the lack of investigation on dopamine D1 and mGlu5 receptor interactions and well-explored regulatory role of adenosine A_1 receptors on dopamine D1 receptor functions, the aim of this study was to evaluate the possible interaction between adenosine A_1 and mGlu5 receptors in modulation of dopamine D1 receptor-induced locomotion in the reserpinised rats. In the present manuscript, we report the effects of co-administration of agents acting upon adenosine A_1 and mGlu5 receptors on the reserpine-treated rat model of Parkinson's disease by evaluating the effects of these compounds on locomotion induced by dopamine D1 receptor agonist.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (250–300 g) were housed in groups of 6 under the controlled conditions (constant temperature 18±1 °C; relative humidity ~30%; 12 h light/dark cycles) and received food and water ad libitum. All studies were performed under a project license according to the UK Animal (Scientific Procedures) Act 1986. Animals were injected with 3 mg/kg reserpine subcutaneously (dissolved in 1% glacial acetic acid in sterile water) under light anaesthesia of Halothane (Sigma, UK). Control groups received only vehicle treatment. Behavioural assessments of the effects of drugs on locomotion were carried out 18 h after the injections. After the completion of each experiment, animals were humanely killed by the overdose of anaesthesia of Halothane.

2.2. Behavioural assessment

The locomotion of animals was assessed by using the automated movement detection system (AM1053, Linton Instrumentation, UK). The system consists of a box and a frame containing infrared beams to detect movement. The light beams are located on two separate independent

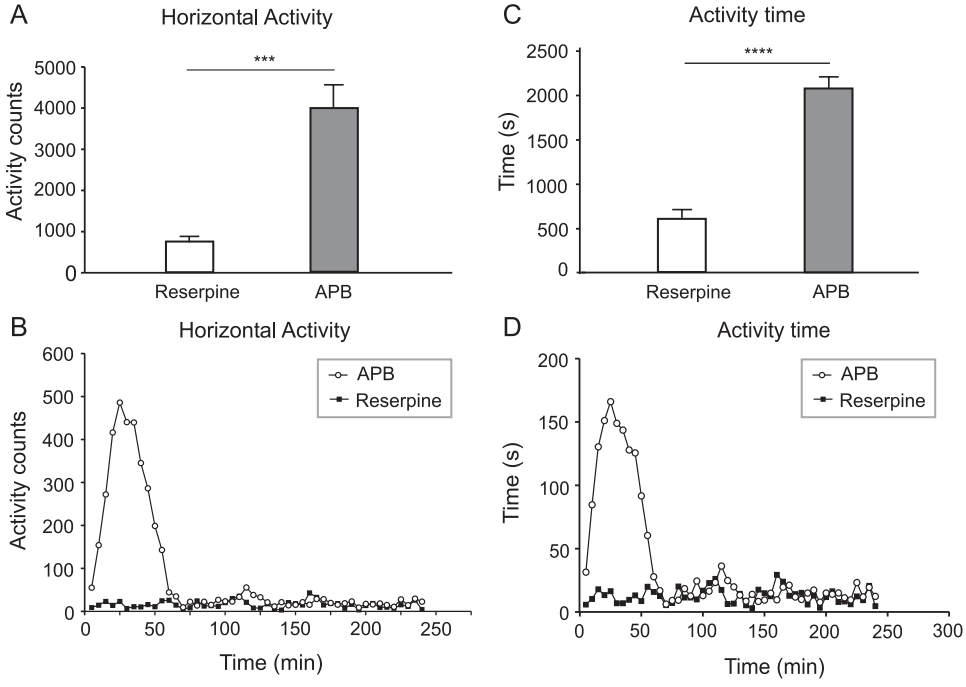

Fig. 1. Effect of administration of 0.2 mg/kg chloro-APB on locomotor behaviour in the reserpine-treated rats. Reserpine (3 mg/kg) was administrated subcutaneously 18 h prior to the behavioural assessment of the animal response to chloro-APB injection. The administration of chloro-APB significantly increased the locomotion activity (A, B) and activity time (C, D) in experimental animals compared to the control group (****Pb0.0001, unpaired Student's t-test, n=6 per group). The locomotion of rats is expressed as activity counts and time (s)±S.E.M.

matrixes, which consist of 16 beams×8 beams on 25-mm grid. The movement detector operates by counting the number of times an animal breaks a beam; in the present study, we measured horizontal activity (the number of beams broken) and activity time (calculated when a beam was broken in the previous second).

Prior to behavioural assessment, animals were acclimatised to the experimental room in locomotion boxes for 30 min before the administration of compounds. After the adaptation, the drugs were administrated intra-peritoneally (i.p.) and the assessment of locomotion commenced immediately. The assessment of rat locomotor activity was carried out for 4 h post injection. Locomotion was measured for each 5 min. The total activity counts were calculated as a summation of the counts observed during 5-min time.

2.3. Pharmacological treatments

All injections were administrated in a volume of 1 ml/kg body weight. Drugs administrated were reserpine (3 mg/kg) (Segovia et al., 2003; Silverdale et al., 2001), chloro-APB (*R*-(±)-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide) or SKF-82958 hydrobromide (0.2 mg/kg), CPA (cyclopentyladenosine, 0.5 mg/kg (Popoli et al., 1997), CPT (8-cyclopentyl-1,3-dimethylxanthine, 0.6 mg/kg (Popoli et al., 1998), MPEP (2-methyl-6-(phenylthyl)pyridine, 10 mg/kg (Anderson et al., 2002). All drugs were dissolved in the water for injections apart from reserpine which was dissolved in 1% acidic acid. The co-administration of compounds was conducted in separate syringes and into different sites of i.p.

2.4. Source of drugs

Reserpine, chloro-APB (dopamine D1 receptor agonist), CPA (adenosine A_1 receptor agonist) and CPT (adenosine A_1 receptor antagonist) were supplied by Sigma-Aldrich (UK); MPEP (mGlu5 receptor antagonist) was purchased by TOCRIS Cookson (UK). Halothane was supplied by Sigma-Aldrich.

2.5. Statistical analysis

The total activity counts and activity time were analysed using unpaired Student's *t*-test for vehicle and reserpine treatment as well as for Figs. 1 and 2 (chloro-APB injections and co-administration of chloro-APB with MPEP). Statistical analysis for the rest of data on pharmacological drug interactions was performed using a one-way analysis of variance (ANOVA) followed by Tukey post hoc test. A significant difference was accepted when the probability level of $Pb0.05$.

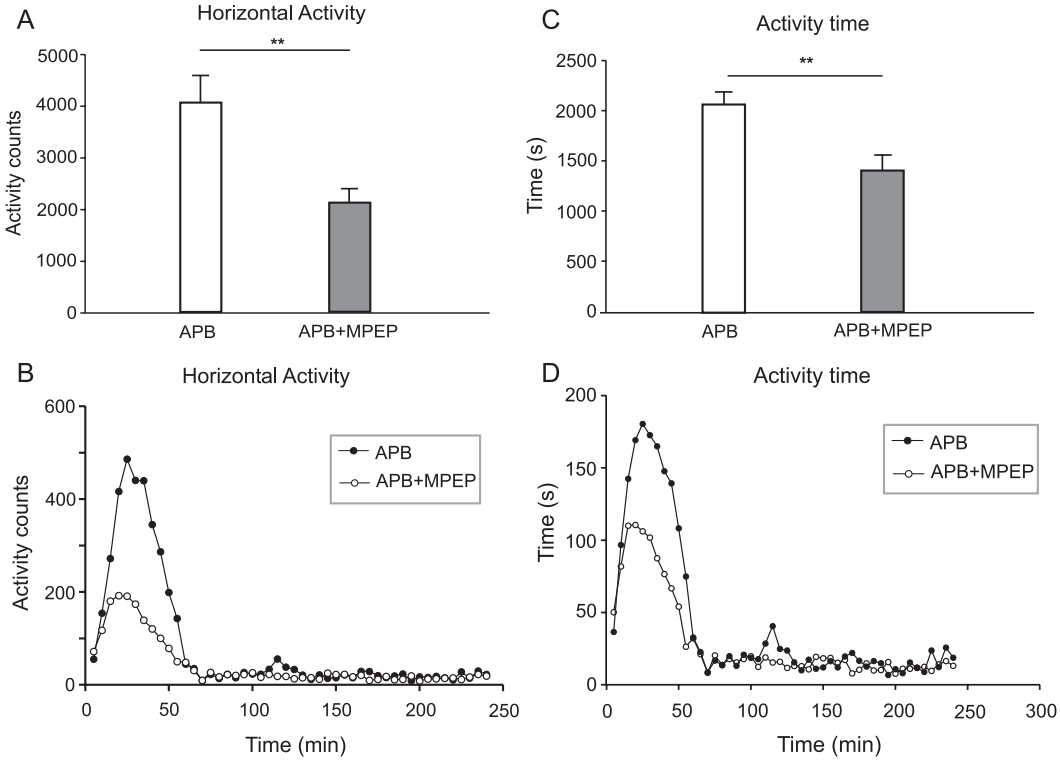

Fig. 2. Effect of the administration 10 mg/kg MPEP, mGlu5 receptor antagonist, on the locomotion behaviour elicited by 0.2 mg/kg chloro-APB in the reserpine-treated rats. Chloro-APB was administrated to the reserpinised rats prior to the behavioural assessment of the animal response to MPEP injection. The administration of MPEP significantly diminished the locomotion activity (A, B) and activity time (C, D) in experimental animals compared to the control group (**$Pb0.01$, unpaired Student's *t*-test, $n=12$ per group). The locomotion of rats is expressed as mobile counts and time (s)±S.E.M.

3. Results

3.1. Effect of reserpine treatment on the motor activity in rats

Eighteen hours after reserpine or vehicle (1% acetic acid) treatment, locomotion activity was dramatically different. The locomotion of vehicle treated animals was characterised by well-coordinated movement: horizontal activity (4727±713 counts) and activity time (2275±272 s). Reserpine treatment resulted in a significant decrease in: horizontal activity 761±85 counts (unpaired Student's *t*-test, n=6 per group, ****Pb0.0001) and activity time 604±103 s, unpaired Student's *t*-test, n=6 per group, ****Pb0.0001) as compared to the vehicle administration in normal animals.

3.2. Effects of administration of MPEP, CPA, CPT alone on parkinsonian state

MPEP, CPA and CPT were administrated 18 h following injection of reserpine. None of the compounds injected on its own produced a significant increase in locomotion, in horizontal activity (vehicle 778±85; MPEP 825±117; CPA 606±99; CPT 685±57 counts, one-way ANOVA, followed by Tukey post hoc test, $F_{3,23}$=0.3515, all PN0.05) nor in activity time (vehicle 624±67 s; MPEP 592±102 s; CPA 505±82 s; CPT 523±36 s, one-way ANOVA, followed by Tukey post hoc test, $F_{3,23}$=0.8508, all PN0.05) as compared to vehicle treatment in hypokinetic rats. The time-courses of the locomotion of reserpinised rats after injections of these compounds were similar. These compounds did not modify the symptoms of the parkinsonian state.

3.3. Effect of chloro-APB treatment on alleviation of reserpine-treated rat hypokinesia

Rats were injected with the dopamine D1 receptor agonist, 0.2 mg/kg chloro-APB, or vehicle (sterile water) 18 h following reserpine administration. Chloro-APB treatment resulted in well-coordinated movement and in a significant increase in both assessment parameters: horizontal activity (4079±520 counts) and activity time (2076±127 s) compared to vehicle treatment (761±129 counts, unpaired Student's *t*-test, n=6 per group ***Pb0.001, Fig. 1A) and 604±103 s, (unpaired Student's *t*-test, n=6 per group, ****Pb0.0001, Fig. 1C), respectively. In general, the effect of dopamine D1 receptor agonist was characterised by animals exhibiting exploratory activity followed by episodes of jumps and stereotypic rearing. The locomotor activity

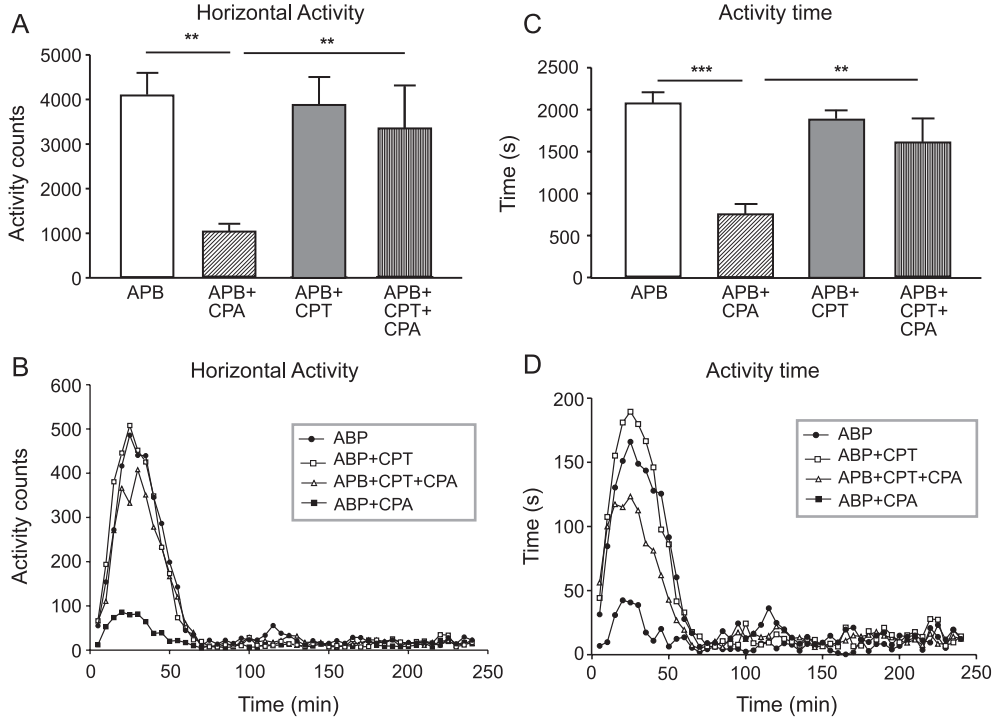

Fig. 3. Effect of the administration of 0.5 mg/kg CPA and 0.6 mg/kg CPT and their combined therapy on the locomotor behaviour elicited by chloro-APB in the reserpine-treated rats. The administration of 0.2 mg/kg chloro-APB was administrated to the reserpinised rats prior to the behavioural assessment of the animal response to CPA and CPT injection and their co-administration. The administration of CPA significantly diminished the locomotion activity (A, B) and activity time (C, D) in experimental animals compared to the control group (**Pb0.001, one-way ANOVA followed by Tukey post hoc test, n=12 per group). The administration of CPT did not exhibit a significantly reduction of the locomotor activity and activity time in experimental animals compared to the control group (PN0.05, one-way ANOVA followed by Tukey post hoc test, n=12 per group), whereas co-administration of CPT and CPA blocked the effect of CPA on dopamine D1 receptor agonist-induced behaviour (**Pb0.001, one-way ANOVA followed by Tukey post hoc test, n=12 per group). The locomotion of rats is expressed as activity counts and time (s)±S.E.M.

induced by chloro-APB lasted approximately 1 h after the injection and peaked by approximately 25 min after the drug administration and then slowly declined (Fig. 1B). The effect of dopamine D1 receptor agonist on activity time had a similar pattern (Fig. 1D).

3.4. Effects of administration of MPEP, CPA and CPT alone and co-administration of CPA and CPT on chloro-APB-induced locomotion in reserpinised rats

There was a significant effect of MPEP on chloro-APB-induced locomotion. Injection of 10 mg/kg MPEP, a mGluR5 antagonist, significantly reduced chloro-APB activity (2098±278 counts compared to chloro-APB-induced locomotion, 4079±520 counts, unpaired Student's *t*-test, n=12 per group, $^{**}Pb0.01$, Fig. 2A,B) and activity time (1417±156 s as compared to the chloro-APB-induced locomotion 2076±127 s, unpaired Student's *t*-test, n=12 per group, $^{**}Pb0.01$ Fig. 2C,D) in reserpinised rats. Similar to MPEP, 0.5 mg/kg CPA, an adenosine A_1 receptor agonist, had a significant effect on chloro-APB-induced activity (997±165 counts, one-way ANOVA, followed by Tukey post hoc test, $F_{3,44}$=5.891, $^{**}Pb0.01$, Fig. 3A,B) and activity time 766±119 s (one-way ANOVA, followed by Tukey post hoc test, $F_{3,44}$=12.38, $^{***}Pb0.001$, Fig. 3C,D). CPT 0.6 mg/kg, an adenosine A_1 receptor antagonist, had no significant effect on the chloro-APB-induced activity (3912±633 counts as compared to chloro-APB above values, one-way ANOVA, followed by Tukey post hoc test, $F_{3,44}$=5.891, $PN0.05$) and activity time (1888±128 s, one-way ANOVA, followed by Tukey post hoc test, $F_{3,44}$=12.38, $PN0.05$) as compared to chloro-APB alone (Fig. 3). However, the combination of adenosine A_1 receptor antagonist successfully blocked the effect of the adenosine A_1 receptor agonist on chloro-APB-induced locomotion: activity 2841±860 counts (one-way ANOVA followed by Tukey post hoc test, $F_{3,44}$=5.891, $^{**}Pb0.01$) and activity time 1608±283 as (one-way ANOVA followed by Tukey post hoc test, $F_{3,44}$=12.38, $^{**}Pb0.01$).

3.5. Effects of co-administration of MPEP, CPA, CPT on chloro-APB-induced locomotion in reserpinised rats

The combination of 0.2 mg/kg chloro-APB, 10 mg/kg MPEP and 0.5 mg/kg CPA significantly reduced the locomotion induced by the dopamine D1 receptor agonist activity: 1461±210 counts (one-way ANOVA, followed by Tukey post hoc test, $F_{3,44}$=17.42, $^{**}Pb0.01$, Fig. 4A,B) and activity time (1076±148 s, one-way ANOVA, followed by Tukey post hoc test, $F_{3,44}$=15.67, $^{**}Pb0.01$, Fig. 4C,D) as compared to chloro-APB above values. There was no significant difference between the effects on animal move-

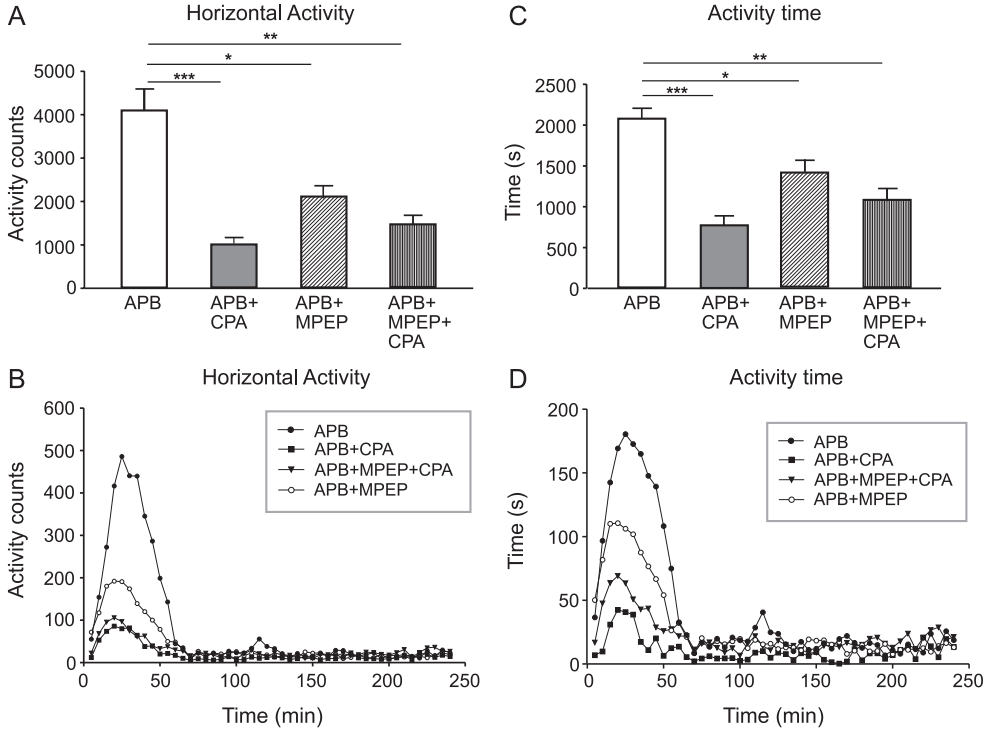

Fig. 4. Effect of the combined therapy of 10 mg/kg MPEP and 0.5 mg/kg CPA on the locomotion elicited by chloro-APB. The administration of 0.2 mg/kg chloro-APB was administrated to the reserpinised rats prior to behavioural assessment of the animal response to CPA and MPEP co-administration. The co-administration of CPA and MPEP significantly diminished the locomotor activity (A, B) and activity time (C, D) in experimental animals compared to the control group ($^{**}Pb0.01$, one-way ANOVA followed by Tukey post hoc test, n=12 per group). The locomotion of rats is expressed as activity counts and time (s)±S.E.M. The co-administration of CPA and MPEP did not exhibit any significant effect as compared to CPA ($PN0.05$, one-way ANOVA followed by Tukey post hoc test) and MPEP ($PN0.05$, one-way ANOVA followed by Tukey post hoc test) treatment alone on locomotion elicited by chloro-APB.

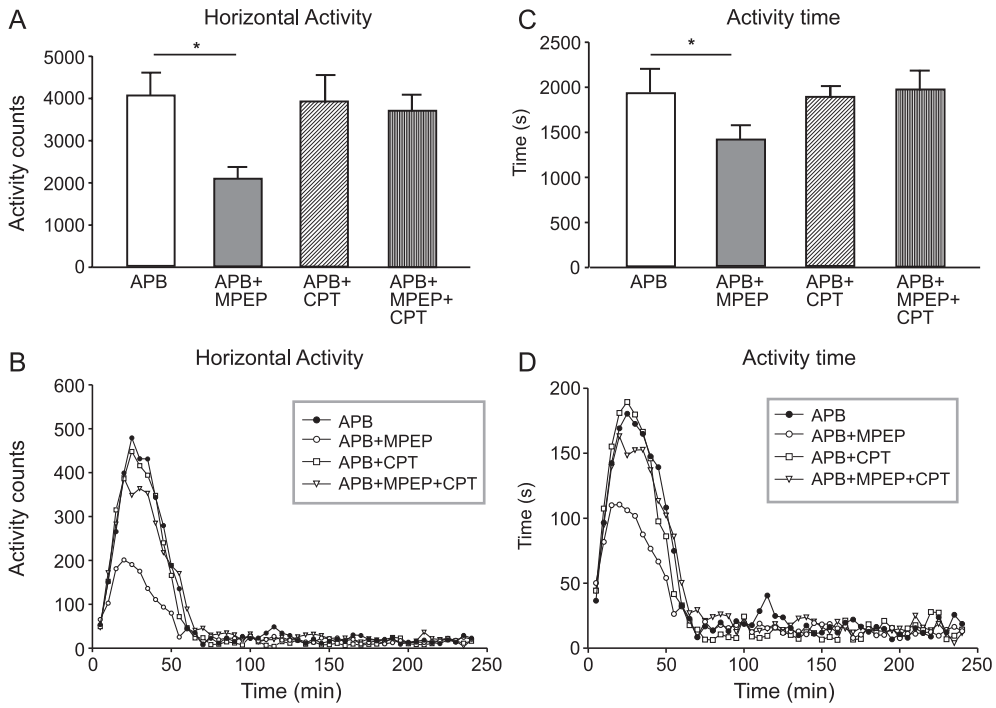

Fig. 5. Effect of the combined therapy of 10 mg/kg MPEP and 0.6 mg/kg CPT on the locomotion elicited by chloro-APB. The administration of 0.2 mg/kg chloro-APB was administrated to the reserpinised rats prior to behavioural assessment of the animal response to CPT and MPEP co-administration. The co-administration of CPT and MPEP did not exhibit a significant change in the locomotor activity (A, B) and activity time (C, D) in experimental animals as compared to the control group ($PN0.05$, one-way ANOVA followed by Tukey post hoc test, n=12 per group). The locomotion of rats is expressed as activity counts and time (s)±S.E.M. The administration of CPT with MPEP blocked the effect of MPEP on locomotion elicited by chloro-APB.

ment of co-administration of MPEP/C1-APB/CPA and co-injection of MPEP with chloro-APB (one-way ANOVA, followed by Tukey post hoc test, $F_{3,44}$=17.42 (15.67), $PN0.05$, Fig. 4A,C).

In contrast, the combination of 0.2 mg/kg chloro-APB, 10 mg/kg MPEP and 0.6 mg/kg CPT did not have any significant effect on chloro-APB-induced activity. The horizontal activity after Cl-APB/MPEP/CPT administration was 3441±446 counts (one-way ANOVA, followed by Tukey post hoc test, $F_{3,44}$=4.050, $PN0.05$, Fig. 5A,B) and activity time 1971±211 s (one-way ANOVA, followed by Tukey post hoc test, $F_{3,44}$=3.695, $PN0.05$, Fig. 5C,D) as compared to chloro-APB administration alone. However, it blocked the effect of MPEP administration on dopamine D1 receptor-induced locomotion.

4. Discussion

This study demonstrates that both the adenosine A_1 receptor agonist (CPA) and the mGlu5 receptor antagonist MPEP can reduce dopamine D1 receptor-induced alleviation of hypokinesia in the reserpine-treated rat model of Parkinson's disease. Furthermore, the inhibitory actions of MPEP on the effects of dopamine D1 receptor stimulation can be attenuated by an adenosine A_1 receptor antagonist (CPT), suggesting that A1 receptor stimulation is required for MPEP to mediate its effects.

Locomotion is controlled by the differential activity of the two GABAergic efferent pathways from the striatum: striatopallidal (the indirect pathway-activation of which inhibits movement) and striatonigral (the direct pathway, activation of which generates movement (Albin et al., 1989; Alexander, 1994)). The regulation of locomotion by both pathways was blocked by the administration of reserpine, which depletes dopamine from neuronal terminals after 18 h of injection (Anden, 1967). In our study, we stimulated locomotion by administration of selective dopamine D1 receptor agonist (Murray and Waddington, 1989; O'Boyle et al., 1989). Dopamine D1 receptor-mediated behavioural changes observed in this model have been interpreted as activation of the direct pathway similarly to previously reported in reserpinised (Popoli et al., 1996b; Segovia et al., 2003) and 6-hydroxydopamine-lesioned rats (Fenu et al., 1995; Fox and Brotchie, 2000; Popoli et al., 2001). However, such an interpretation is taken with caution as other potential sites of dopamine D1 receptor agonist action in the brain (e.g. the subthalamic nucleus (Hemsley et al., 2002) or the substantia nigra (Hemsley and Crocker, 2001)) could be involved in the motor alleviation of hypokinetic state following the intraperitoneal administration of this compound.

Interestingly, activation of the direct pathway is thought to underlie the generation of L-DOPA-induced dyskinesia (Bezard et al., 2001). Thus, while the behaviour elicited by the dopamine D1 receptor agonist in reserpinised rats was

not equivalent to L-DOPA-induced dyskinesia, the two locomotor activities may share a common neuronal mechanism, and this model may be useful for defining both mechanisms and new treatments of this disorder. Indeed, it has previously shown that agents that reduce dopamine D1 receptor-agonist-induced locomotion in reserpinised rats can reduce dyskinesia (Segovia et al., 2003).

During the last decade, the pharmacology of communication between dopamine and metabotropic glutamate 5 receptors has attracted a significant attention (Diaz-Cabiale et al., 2002; Parelkar and Wang, 2003; Popoli et al., 2001). Our data suggest that the blockade of mGlu5 receptors could have a beneficial effect in L-DOPA-induced dyskinesia by countering dopamine D1 receptor activation of the direct pathway. The administration of MPEP alone to reserpine-treated rats did not exhibit any significant alterations in locomotor activity, suggesting that its inhibition of chloro-APB-induced locomotion was limited to dopamine D1 receptor stimulation. Therefore, MPEP as other mGluR5 antagonists might effectively represent a pharmacological tool in anti-dyskinetic management.

Antagonistic interaction between adenosine A_1 and dopamine D1 receptor-activating compounds shown in our study is consistent with the previous observations in reserpinised animals (Ferre et al., 1994; Popoli et al., 1996b). We also believe that the effects of the adenosine A_1 receptor agonist (CPA) and antagonist (CPT) are limited to dopamine D1 receptor-stimulated locomotor activity since they cannot modify the motor effects of dopamine D2-receptor-modulating compounds (Ferre et al., 1994; Fuxe et al., 1998; Popoli et al., 1996b). Adenosine A_1 receptor agonist, CPA, on its own exhibited a powerful inhibitory effect on locomotion induced by the chloro-APB administration. However, the adenosine A_1 antagonist, CPT, did not further increase dopamine D1 receptor-agonist-induced motor activity in reserpinised rats as was shown in previous studies of Popoli et al. (1996b). In our study, we employed a full dopamine D1 receptor-agonist, chloro-APB, which has a full stimulatory effect on dopamine D1 receptors (Murray and Waddington, 1989; O'Boyle et al., 1989), than SKF 38393, a partial agonist with 70% stimulatory effect, used in the previous study.

The data presented here suggest that there is a functional interaction between mGlu5 and adenosine A_1 receptors with regard to locomotion generated by dopamine D1 receptor activation. Thus, the inhibitory effect of MPEP on dopamine D1 receptor stimulation can be blocked by an adenosine A_1 receptor antagonist. Since administration of the adenosine A_1 receptor antagonist (CPT) did not influence the effect of dopamine D1 receptor stimulation, the ability of CPT to counteract the inhibitory effect of MPEP on locomotion elicited by chloro-APB could be attributed to a functionally antagonistic interaction between mGlu5 and adenosine A_1 receptor blockade rather than similar independent effects of both on dopamine D1-receptor-mediated behaviour. A corollary of this would be that stimulation of adenosine A_1 receptors would enhance the effects of MPEP. In fact, while we were able to demonstrate the previously described inhibitory effects of adenosine A_1-receptor stimulation on behaviours elicited by dopamine D1 receptor agonist (Ferre et al., 1999; Rimondini et al., 1998), CPA did not significantly increase the inhibitory effect of MPEP on chloro-APB stimulated locomotor activity. Our inability to demonstrate an interaction between the mGlu5 receptor antagonist and adenosine A_1 receptor agonist could be explained by reaching a *d*floor effect*T* whereby, at the doses employed, both compounds are exerting their maximal effects and so demonstration of additivity or synergy was beyond the power of the study, as designed. It is tempting to speculate that the site of action of the modulatory control of mGlu5 receptors over the dopamine D1 receptor regulated activity is at the level of the striatonigral pathway and might thus represent an effective pharmacological tool in the management of overactivity of this pathway in the L-DOPA-induced dyskinesia. However, it must be appreciated that MPEP can also act on other sites of the brain, including motor cortex and other basal ganglia regions (Tallaksen-Greene et al., 1998; Testa et al., 1994, 1995), which also contribute to the modulation of locomotor activity (Alexander, 1994; Smith et al., 1998).

On the basis of the data presented, we could speculate that the stimulation of adenosine A_1 receptor underlies or is needed for the actions of MPEP. For instance, the blockade of mGlu5 receptors may decrease the effect of dopamine D1 receptor activation by stimulating adenosine A_1 receptors or by recruiting some processes upon which adenosine A_1 receptor stimulation is permissive. Several reports (e.g. Basheer et al., 2002; Cormier et al., 2001) show that adenosine acting through adenosine A_1 receptors affects phospholipase C (PLC) activity (Biber et al., 1997) and modulates the intracellular calcium via inositol-1,4,5-phosphate (InsP3) dependent pathway. Since Group I mGlu receptors, including mGlu5 receptors, also operate through the PLC/InsP3 signalling pathway (Alberts et al., 1994; Dale et al., 2002; Nakamura et al., 2000; Peavy et al., 2002), it could be suggested that mGlu5 and adenosine A_1 receptors might interact on this level of signalling. However, bearing in mind that our study was based on an animal behavioural response to different compounds administered systemically, investigation of mGlu5–adenosine A_1 receptor interactions at the level of intracellular signalling cascades is necessary to substantiate such speculations.

Our data are consistent with the hypothesis of cross-talk between dopamine D1, adenosine A_1 and metabotropic glutamate 5 receptor-modulating compounds. We suggest that such an action may take place in the striatum. Moreover, our data highlight the opportunities of CPA and MPEP to be employed in the treatment of basal ganglia related disorders, such as L-DOPA-induced dyskinesia, though further studies will be required in validated rodent and primate models.

Acknowledgements

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