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# New adenosine $A_{2A}$ receptor antagonists: Actions on Parkinson's disease models

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**Abstract**

The 8-substituted 9-ethyladenine derivatives: 8-bromo-9-ethyladenine (ANR 82), 8-ethoxy- 9-ethyladenine (ANR 94), and 8-furyl-9-ethyladenine (ANR 152) have been characterized in vitro as adenosine receptor antagonists. Adenosine is deeply involved in the control of motor behaviour and substantial evidences indicate that adenosine $A_{2A}$ receptor antagonists improve motor deficits in animal models of Parkinson's disease. On this basis, the efficacy of ANR 82, ANR 94, and ANR 152 in rat models of Parkinson's disease was evaluated. All compounds tested reversed the catalepsy induced by haloperidol. However, in unilaterally 6-hydroxydopamine-lesioned rats, only ANR 94 and ANR 152 potentiated L-dihydroxy-phenylalanine (L-DOPA) effect on turning behaviour and induced contralateral turning behaviour in rats sensitised to L-DOPA. Taken together the results of this study indicate that some 8-substituted 9-ethyladenine derivatives ameliorate motor deficits in rat models of Parkinson's disease, suggesting a potential therapeutic role of these compounds.


*Keywords:* Adenosine $A_{2A}$ antagonist; Parkinson's disease; Dopamine; 6-hydroxydopamine; L-DOPA (L-dihydroxy-phenylalanine)

## 1. Introduction

Parkinson's disease is characterized by a motor impairment caused by the degeneration of dopaminergic neurons located in the substantia nigra pars compacta and by the reduction of dopamine levels in the striatum. Replacement therapy with the dopamine precursor L-dihydroxy-phenylalanine (L-DOPA) is successful in most parkinsonian patients. After several years of L-DOPA therapy, however, many motor complications, including fluctuations in motor responses and dyskinesia occur (Kopin, 1993). Therefore, alternative therapeutic approaches are the target of active research in Parkinson's disease.

Adenosine $A_{2A}$ receptors are predominantly located in the striatum (Jarvis and Williams, 1989; Rosin et al., 1998; Svenningsson et al., 1999), where they are co-expressed with dopamine $D_2$ receptors on the indirect striatopallidal pathway (Fink et al., 1992; Schiffmann et al., 1991). Stimulation of adenosine $A_{2A}$ receptors decreases the binding affinity of dopamine for dopamine $D_2$ receptors (Dasgupta et al., 1996; Ferré et al., 1991) and elicits effects opposite to dopamine $D_2$ receptor activation at the level of second messenger systems and early-gene expression (Le Moine et al., 1997; Morelli et al., 1995). However, stimulation, as well as blockade of adenosine $A_{2A}$ receptors, induces behavioral and biochemical responses in dopamine $D_2$ receptor knockout mice, suggesting that adenosine $A_{2A}$ receptor actions can occur independently from dopamine (Aoyama et al., 2000; Chen et al., 2001; Zahniser et al., 2000).

Blockade of adenosine $A_{2A}$ receptors produces motor stimulant effects (El Yacoubi et al., 2000; Griebel et al., 1991; Hauber et al., 1998; Holtzman, 1991) and reverses catalepsy induced by dopamine receptor blockade or by dopamine depletion (Kanda et al., 1994; Mandhane et al., 1997; Shiozaki et al., 1999; Wardas et al., 2001). Moreover, studies in experimental 1-methyl-4-phenyl-1, 2, 3, 6-

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tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) models of Parkinson's disease have shown that adenosine $A_{2A}$ receptor antagonists have positive effects on motor impairments and potentiate dopamine agonist-stimulated motor behaviour (Fenu et al., 1997; Grondin et al., 1999; Jiang et al., 1993; Kanda et al., 1998; Pinna et al., 1996; Pollack and Fink, 1996). Interestingly, recent clinical evidences showed that adenosine $A_{2A}$ receptor antagonists are effective in reducing motor impairment in parkinsonian patients with low risk of dyskinesias (Chase et al., 2003; Kase et al., 2003; LeWitt, 2004).

Recently, a class of 9-ethyladenine derivatives has been characterized as adenosine receptor ligands (Camaioni et al., 1998). Among them, the 8-bromo-9-ethyladenine (ANR 82), showed high affinity and moderate selectivity for the human adenosine $A_{2A}$ receptor subtype (Volpini et al., 2003). In order to improve the $A_{2A}$ binding affinity and selectivity of ANR 82, the 8-bromine atom was replaced with an ethoxy or a furyl substituent, to form the compound 8-ethoxy-9-ethyladenine (ANR 94) (Klotz et al., 2003) and 8-furyl-9-ethyladenine (ANR 152). These two compounds showed higher selectivity and affinity, respectively, than the origin compound.

In this study, the in vivo activity of these three derivatives was investigated by assessing the ability of reversing locomotor deficits induced by haloperidol or inducing contralateral turning behaviour in rats bearing an unilateral 6-OHDA lesion of the nigrostriatal pathway (Ungerstedt, 1971), two rodent models largely used to evaluate the efficacy of antiparkinson drugs. In 6-OHDA-lesioned rats, both potentiation of contralateral rotation induced by a subthreshold dose of L-DOPA and induction of contralateral turning, in rats previously sensitized to the motor effect of L-DOPA, were evaluated.

## 2. Materials and methods

### 2.1. Animals

Male Sprague–Dawley rats (Charles River, Italy) weighing 275–300 g were used. Rats were housed in groups of five with free access to food and water and maintained in a temperature-controlled (22 ± 1 C) and light-controlled room (12-h light/dark cycle). Testing was conducted during the light phase. The guidelines of the European Community for animal experiments were followed (86/609/EEC; D.L., 27.01.1992, number 116).

### 2.2. Catalepsy testing

Catalepsy was estimated through the vertical grid test. The test was carried out by placing the rat with the four paws on a wire grid (43 × 25 cm) at an angle of about 70° respect to the bench surface. Catalepsy was determined by measuring the time in which the rat maintained the given position. Test was terminated when the rat moved one paw or when 90 s had elapsed from the placement of the rat on the grid.

Catalepsy assessments were repeated every 10- to 15-min intervals. At each test time, rats which did not assume the given position on the grid after three attempts were classified 0 s latency. Adenosine $A_{2A}$ receptor antagonists were injected 90 min after haloperidol administration.

### 2.3. 6-hydroxydopamine lesion

Rats were anaesthetised with chloral hydrate (400 mg/kg i.p.), placed in a David Kopf stereotaxic apparatus and injected, through a stainless steel cannula in the left medial forebrain bundle with 6-OHDA–HCl (8 μg/4 μl of saline containing 0.05% ascorbic acid), at coordinates AP = −2.2, ML = +1.5, DV = −7.8, according to the atlas of Pellegrino et al. (1979). Rats were pretreated with desipramine (10 mg/kg i.p.) in order to prevent 6-OHDA-induced neurotoxicity to noradrenergic neurons.

### 2.4. Evaluation of turning behaviour

#### 2.4.1. Potentiation of L-DOPA-induced turning behaviour

Two weeks after the unilateral 6-OHDA lesion, rats were screened on the basis of their contralateral rotation in response to L-DOPA (50 mg/kg i.p.) + benserazide (30 mg/kg i.p.). Rats not showing at least 300 contralateral rotations during the 2-h testing period were eliminated from the study. Three days later, rats were administered with L-DOPA (3 mg/kg i.p.) + benserazide (6 mg/kg i.p.) in combination with vehicle or with a dose of 5 mg/kg i.p of ANR 82, ANR 94, or ANR 152. ANR compounds were administered at the same time of L-DOPA. Benserazide was always injected 30 min before L-DOPA.

In order to measure turning behaviour, rats were placed in plexiglas hemispherical bowls (50 cm of diameter) 30 min before the administration of benserazide to acclimatize and the number of both contralateral and homolateral rotations were counted by automated rotameters, every 10 min, for 2 h.

#### 2.4.2. Turning behaviour induced by ANR 82, ANR 94, and ANR 152

Two weeks after the 6-OHDA infusion, rats were primed one or four times with L-DOPA. Rats were, therefore, injected twice a week (3-day interval) for 2 weeks with vehicle or L-DOPA (6 mg/kg i.p.) + benserazide (6 mg/kg i.p.). As shown by Fenu and Morelli (1998), this drug treatment sensitised rats to adenosine antagonist-induced turning behaviour. Rats not showing at least 150 contralateral rotations during the first 2-h testing period in the first administration were eliminated from the study. Three days after the end of the single or four times priming procedure, rats were injected with a dose of 5 mg/kg i.p. of ANR 82, ANR 94, or ANR 152. The number of both contralateral and homolateral rotations were counted by automated rotameters, every 10 min, for 2 h.

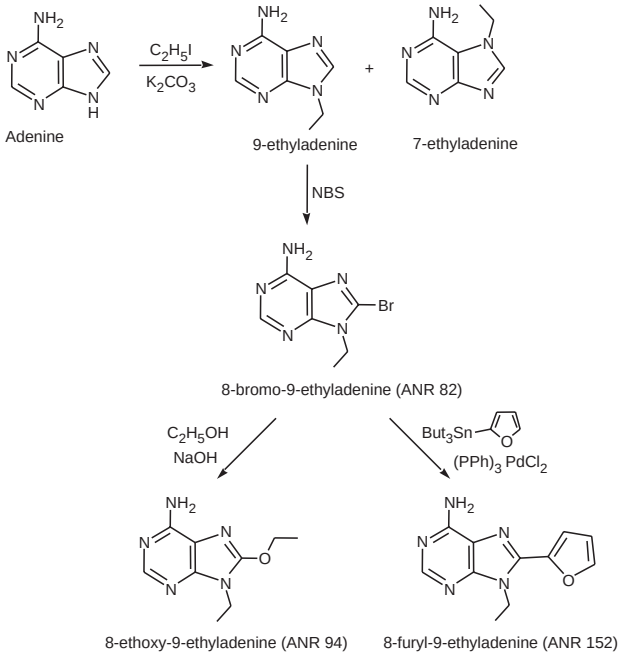


Fig. 1. Chemical structures of ANR 82, ANR 94, and ANR 152. NBS=*N*-bromosuccinimide.

### 2.5. Drugs

The 8-substituted 9-ethyladenine derivatives, ANR 82, ANR 94, or ANR 152, were synthesized starting from commercially available adenine, which was alkylated using ethyl iodide in the presence of potassium carbonate. Both the 9-ethyladenine and its N-7 isomer were obtained and the two isomers were separated through chromatography and the structure was assigned to each compound using spectrophotometric techniques (Fig. 1).

The 9-ethyladenine was treated with *N*-bromosuccinimide (NBS) to obtain the first final product: the 8-bromo-9-ethyladenine (ANR 82; Camaioni et al., 1998). Reaction of ANR 82 with ethanol in the presence of sodium hydroxide produced the 8-ethoxy-9-ethyladenine (ANR 94), while the 8-furyl-9-ethyladenine (ANR 152) was obtained by treating ANR 82 with tributylstannylfuran. The synthesis of these two compounds will be reported elsewhere (In publication).

ANR 82, ANR 94 and ANR 152 were dissolved by adding dimethylsulfoxide (DMSO), polyethylene glycol (PEG 400) and water of the ratio (50:350:600), and vortexing vigorously; the clear solution was injected in a volume of 0.3 ml i.p. per 100 g body weight.

6-OHDA–HCl, desipramine, benserazide, and L-DOPA were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Haloperidol was purchased from commercial source (Serenase, Lusofarmaco, Italy), diluted in distilled water, and administered s.c. The drugs administered parenterally were dissolved in saline and injected in a volume of 0.3 ml i.p. per 100 g body weight or in a volume of 0.1 ml s.c. per 100 g body weight.

### 2.6. Data analysis and statistic

For catalepsy evaluation, the mean of total time spent by rats in immobility during each test section and S.E.M. were calculated. Significance between groups was evaluated by one way analysis of variance (ANOVA) followed by a Newman–Keuls post hoc test.

In the turning behaviour experiments, mean and S.E.M. of the total number of rotations were calculated. Significance between groups was evaluated by one-way ANOVA followed by a Newman–Keuls post hoc test.

## 3. Results

As shown by previous studies (Klotz et al., 1998, 2003) ANR 82, ANR 94, and ANR 152 have been evaluated in human recombinant adenosine receptors, stably transfected into Chinese hamster ovary (CHO) cells, utilizing radioligand binding studies ($A_1$, $A_{2A}$, $A_3$), or adenylyl cyclase activity assay ($A_{2B}$). Receptor binding affinity was determined using [$^3$H]CCPA (2-chloro-$N^6$-cyclopentyladenosine) as radioligand for adenosine $A_1$ receptors, whereas [$^3$H]NECA (5′-*N*-ethylcarboxamidoadenosine) was used for binding adenosine $A_{2A}$ and adenosine $A_3$ receptor subtypes. The results of those studies are shown in Table 1.

The 8-ethoxy derivatives ANR 94 showed affinity versus the adenosine $A_{2A}$ receptor comparable to that of ANR 82, while it resulted more selective than ANR 82 at the adenosine $A_{2A}$ receptor (ANR 94 $A_1/A_{2A}$ ratio=52, $A_{2B}/A_{2A}$=652, and $A_3/A_{2A}$=456; ANR 82 $A_1/A_{2A}$ ratio=5, $A_{2B}/A_{2A}$=16, and $A_3/A_{2A}$=534; Table 1). The substitution of the bromine atom with a furyl ring, led to a compound, ANR 152, endowed with affinity higher than ANR 82 at all

Table 1
Affinity of 8-substituted 9-ethyladenines at human adenosine receptor subtypes

| Compound | PM | $A_1$ | $A_{2A}$ | $A_{2B}$ | $A_3$ |
|---|---|---|---|---|---|
| ANR 82 | 242.1 | 280 (250–320) | 52 (24–113) | 840 (630–1120) | 27,800 (22,300–34,700) |
| ANR 94 | 207.2 | 2400 (2100–2600) | 46 (24–91) | >30,000 | 21,000 (11,100–41,000) |
| ANR 152 | 229.2 | 24 (16–34) | 3.7 (3.0–4.6) | 380 (250–560) | 4700 (2900–7600) |

$K_i$ values for adenosine $A_1$ receptors are from competition experiments with [$^3$H]CCPA, for adenosine $A_{2A}$ and adenosine $A_3$ receptors [$^3$H]NECA was used as a radioligand. In the case of adenosine $A_{2B}$ receptors $K_i$-values were calculated from $IC_{50}$-values determined by inhibition of NECA-stimulated adenylyl cyclase activity. $K_i$ values are in nM with 95% confidence intervals in parentheses.

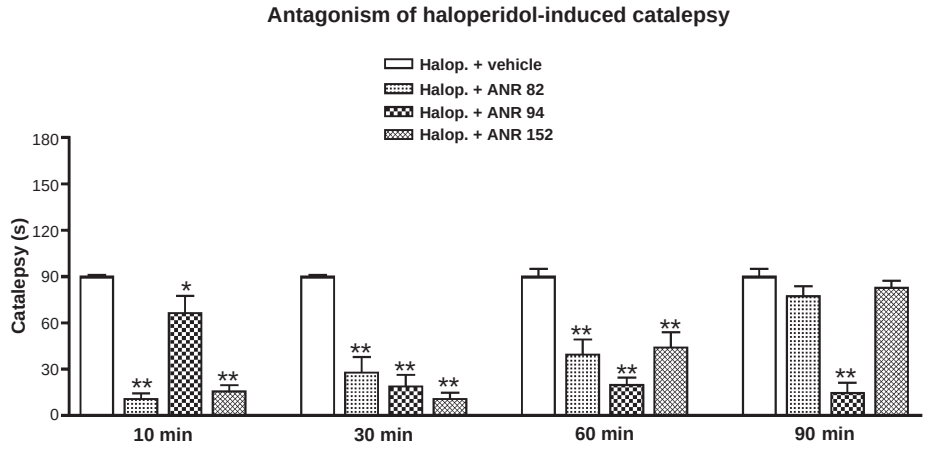


Fig. 2. Effect of ANR 82 (5 mg/kg i.p.; $n=8$), ANR 94 (5 mg/kg i.p.; $n=5$), or ANR 152 (5 mg/kg i.p.; $n=9$) on catalepsy induced by haloperidol (0.2 mg/kg s.c.; $n=7$) in rats, 10, 30, 60, and 90 min after drug administration. Results are mean ± S.E.M. of intensity of catalepsy measured as time spent on cataleptic posture by each rat in test section. $^{**}p<0.0001$, $^{*}p<0.05$ versus haloperidol + vehicle. Statistical significance was determined by one-way ANOVA followed by Newman–Keuls post hoc test.

adenosine receptors, making this compound the most active of the series (Table 1).

### 3.1. Catalepsy

At the dose of 5 mg/kg i.p. the adenosine $A_{2A}$ receptor antagonists ANR 82, ANR 94 and ANR 152 did not modify spontaneous motility in rats, whereas at higher doses (10, 15 mg/kg) they induced hypermotility (data not shown). Therefore, in order to prevent non specific interference with catalepsy the dose of 5 mg/kg i.p. of the adenosine $A_{2A}$ receptor antagonists was used in this study.

Furthermore, the dose of 5 mg/kg i.p. of the adenosine $A_{2A}$ receptor antagonists used in this study was chosen on the basis of preliminary studies showing that 1 mg/kg of ANR 82, ANR 94, or ANR 152 had low efficacy on catalepsy, whereas 5 mg/kg was fully effective.

Haloperidol (0.2 mg/kg s.c.) produced significant catalepsy in rats at 40 min after drug administration and reached the maximum at 60–70 min (data not shown). Therefore, the administration of the new adenosine $A_{2A}$ receptor antagonists was made 90 min after haloperidol, in order to evaluate their effects on deeply cataleptic rats.

At a dose of 5 mg/kg i.p. ANR 82, ANR 94, or ANR 152 significantly reversed the catalepsy induced by 0.2 mg/kg of haloperidol during the 90-min testing period (Fig. 2). The effect of ANR 82 and ANR 152 was maximal at 10–30 min, whereas the effect of ANR 94 was maximal at 30–60 min.

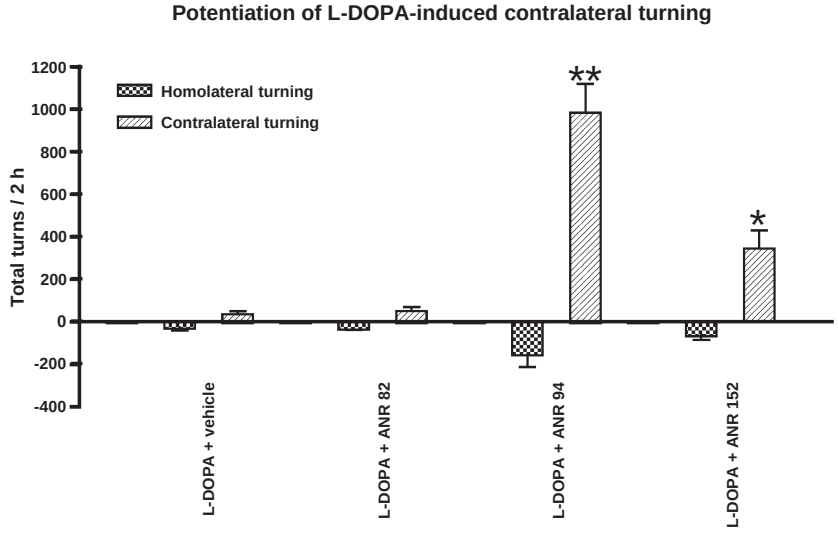


Fig. 3. Effect of administration of L-DOPA (3 mg/kg i.p.) + vehicle ($n=5$), L-DOPA (3 mg/kg i.p.) + ANR 82 (5 mg/kg i.p.; $n=4$), L-DOPA (3 mg/kg i.p.) + ANR 94 (5 mg/kg i.p.; $n=6$), or L-DOPA (3 mg/kg i.p.) + ANR 152 (5 mg/kg i.p.; $n=6$). Ordinate indicates the total number of turns measured in 2 h; positive and negative values represent contralateral and homolateral rotations, respectively. Results are mean + S.E.M. of total turns. $^{**}p<0.0001$, $^{*}p<0.05$ versus L-DOPA alone. Statistical significance was determined by one-way ANOVA followed by Newman–Keuls post hoc test.

The duration of the anticataleptic effect of ANR 82 and ANR 152 was similar lasting about 80 min (Fig. 2). In contrast, the anticataleptic effect of ANR 94 had a longer duration, over 150 min (data not shown). As shown in Fig. 2, rats did not return cataleptic within the 90-min testing.

### 3.2. Potentiation of L-DOPA-induced turning behaviour

Administration of ANR 82 failed to increase the number of contralateral rotations induced in 6-OHDA-lesioned rats by a subthreshold dose of L-DOPA (3 mg/kg i.p.; Fig. 3). In contrast, both ANR 94 and ANR 152, significantly increased the number of contralateral rotations induced by L-DOPA (3 mg/kg) in 6-OHDA-lesioned rats (Fig. 3). The increase in the number of rotations produced by ANR 152 was less marked than that observed with ANR 94 (Fig. 3). Similarly to the anticataleptic effect, the potentiation of ANR 152 on L-DOPA-induced turning behaviour lasted about 80 min, whereas the effect of ANR 94 effect lasted up to 120–130 min. Moreover, after ANR 94+L-DOPA administration the contralateral turns increased to a maximum of 148 ± 20 turns at 40 min, whereas contralateral turns produced by ANR 152+L-DOPA reached a maximum of 85 ± 20 at 30 min. Therefore, the most pronounced effect of ANR 94 on L-DOPA-induced turning behaviour appears to be due to both stronger efficacy and longer duration.

Administration of ANR 94 and ANR 152 resulted in few homolateral turns (Fig. 3).

### 3.3. Turning behaviour in L-DOPA-sensitised rats

In order to evaluate the ability of the new adenosine $A_{2A}$ receptor antagonists to induce contralateral turning behaviour by themselves, different groups of rats received one or four administrations of L-DOPA (6 mg/kg i.p.) and 3 days later they were treated with ANR 82, ANR 94 and ANR 152.

Administration of ANR 94 (5 mg/kg) or ANR 152 (5 mg/kg) produced significant contralateral rotation in rats that received four L-DOPA (6 mg/kg) primings (Fig. 4). On the other hand, rats with a single L-DOPA priming, did not rotate in response to 5 mg/kg of ANR 94 or ANR 152 (Fig. 4). In contrast, ANR 82 (5 mg/kg) failed to induce significant contralateral turning in either four or single L-DOPA-primed rats (Fig. 4).

The effect of ANR 152 on turning behaviour was extinguished 80 min after administration, whereas the effect of ANR 94 lasted up to 150 min.

Both ANR 94 and ANR 152 induced a low intensity homolateral turning behaviour during the 2-h testing (Fig. 4).

## 4. Discussion

The results of this study indicate that the new adenosine $A_{2A}$ receptor ligands 8-substituted 9-ethyladenine derivates are effective in two rat models of Parkinson's disease: the haloperidol catalepsy reversal and the 6-OHDA model of contralateral turning behaviour.

Considering that stimulation, or blockade of adenosine receptors potentiates and reverses haloperidol-induced catalepsy, respectively (Kanda et al., 1994; Mandhane et al., 1997; Shiozaki et al., 1999), the effects of the 8-substituted 9-ethyladenine derivatives were at first investigated in this experimental paradigm in order to confirm that binding to adenosine $A_{2A}$ receptors resulted in functional antagonistic actions on this receptor. All three compounds investigated, reversed haloperidol-induced cata-

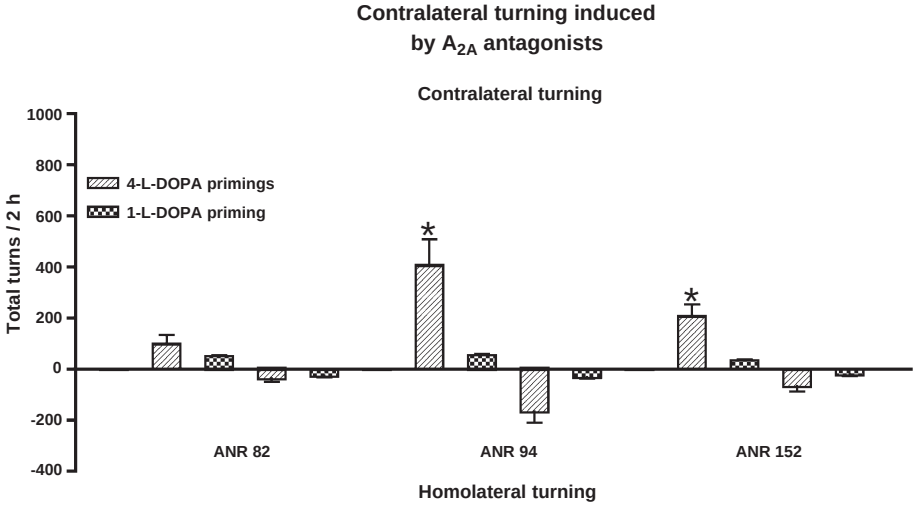


Fig. 4. Total rotations after ANR 82 (5 mg/kg i.p.; $n=7$), ANR 94 (5 mg/kg i.p.; $n=5$), or ANR 152 (5 mg/kg i.p.; $n=5$) in rats primed with one (squared columns) or four (dashed columns) administrations of benserazide (6 mg/kg i.p.)+L-DOPA (6 mg/kg i.p.) 2 weeks after 6-OHDA lesioning. Compounds were administered 3 days after priming. Ordinate indicates the total number of turns measured in 2 h; positive and negative values represent contralateral and homolateral rotations, respectively. Results are mean+S.E.M. of total turns. *$p<0.05$ versus single priming. Statistical significance was determined by one-way ANOVA followed by Newman–Keuls post hoc test.

lepsy, thus showing a pharmacological profile similar to that of proved adenosine $A_{2A}$ receptor antagonists (Kanda et al., 1994; Mandhane et al., 1997; Shiozaki et al., 1999). The assessment of the anticataleptic effect revealed some differences among the derivatives examined. ANR 82 and ANR 152 were maximally effective right after their administration and their action lasted for about 80 min. In contrast, the effect of ANR 94 showed a slower onset but a longer duration as compared to ANR 82 and ANR 152.

On the basis of the ability in reversing catalepsy showed by the 8-substituted 9-ethyladenine derivatives, accounting for a functional antagonism at adenosine $A_{2A}$ receptors, the effects of these compounds were further investigated using unilaterally 6-OHDA-lesioned rats.

Numerous experimental evidences indicate that adenosine $A_{2A}$ receptor antagonists potentiate the contralateral turning induced by a subthreshold dose of L-DOPA (Fenu et al., 1997; Koga et al., 2000; Pinna et al., 2001). Administration of ANR 94, as well as ANR 152, significantly potentiated L-DOPA-induced rotational behaviour, whereas ANR 82 was not effective in this test. Furthermore, ANR 94 and ANR 152 were able to induce contralateral turning in L-DOPA-sensitised rats even without concomitant administration of L-DOPA. The positive response on turning behaviour predicts that ANR 94 and ANR 152, similarly to other selective adenosine $A_{2A}$ receptor antagonists, might have antiparkinsonian effects. The higher efficacy of ANR 94 as compared to ANR 152 might be due to its higher selectivity toward adenosine $A_{2A}$ receptors, whereas the longer duration might be related to its longer half-life, although pharmacokinetic studies on these compounds have not been performed yet.

The results obtained with these new synthesis adenosine $A_{2A}$ receptor antagonists, show that ANR 82 although antagonised catalepsy induced by haloperidol was not effective in the turning behaviour tests. The origin for this difference are not clear, however, since haloperidol catalepsy is due to a selective acute dopamine $D_2$ receptor blockade, whereas contralateral turning induced by L-DOPA is due to stimulation of both dopamine $D_1$ and $D_2$ receptors, one might envision in these different mechanisms the basis of this discrepancy. ANR 82 might, in fact, induce reversal of dopamine $D_2$-mediated catalepsy through the functional antagonistic interaction with dopamine $D_2$ receptors, which are coexpressed on striatopallidal neurons with adenosine $A_{2A}$ receptors (Fink et al., 1992; Schiffmann et al., 1991). This antagonistic effect involves the interaction between the adenosine $A_{2A}$ and dopamine $D_2$ receptors at both receptors and second messenger level (Dasgupta et al., 1996; Ferré et al., 1991); although, an interaction at the level of cholinergic or γ-aminobutyric acid (GABA)ergic neurons, whose release is controlled by adenosine $A_{2A}$ receptors, cannot be excluded (Kurokawa et al., 1996; Mori and Shindou, 2003; Ochi et al., 2000). At the same time, ANR 82 by binding non-adenosine receptors, which interact with dopamine $D_1$ receptors (e.g. serotonin 5-$HT_2$ receptors), might contrast the potentiation of L-DOPA and produce no increase of L-DOPA contralateral turning or contralateral turning in L-DOPA-sensitised rats (Bishop and Walker, 2003).

Influences of adenosine $A_{2A}$ receptors on dopamine $D_1$-mediated behaviours have been explained by functional antagonistic influences of striatopallidal and striatonigral pathways on output structures (Ferré et al., 1997; Hauber et al., 2001; Pinna et al., 1996). Further studies on binding activity of these compounds might clarify this issue; however, the effectiveness of ANR 82 in the catalepsy paradigm deserves interest since ANR 82 is the precursor of ANR 94 and ANR 152.

Interestingly, our results, show that the adenosine $A_{2A}$ receptor antagonists ANR 94 and ANR 152, differently from other selective adenosine $A_{2A}$ receptor antagonists like SCH 58261 or KW 6002, produce contralateral turning in L-DOPA-primed rats even when are administered alone, without concomitant administration of L-DOPA. This result suggests that this class of adenosine $A_{2A}$ receptor antagonists, in the presence of dopamine receptor supersensitivity, like after repeated L-DOPA treatment, might be effective as monotherapy.

Previous studies have suggested that dopamine deficits produce modification in adenosine transmission (Ekonomou et al., 2004; Pinna et al., 2002) facilitating adenosine $A_{2A}$ receptors activity (Morelli et al., 1995). Since adenosine $A_{2A}$ receptor stimulation plays a negative role in the control of motor behaviour (Hauber and Munkle, 1997; Morelli et al., 1994; Rimondini et al., 1997), blockade of adenosine $A_{2A}$ receptors has been suggested to be a useful therapeutic strategy for increasing the efficacy of dopamine receptor agonists like L-DOPA (Kase et al., 2003; Morelli, 2003; Richardson et al., 1997). Complications in chronic L-DOPA-treated parkinsonian patients appear to be due to the advanced dopamine neuron degeneration as well as the chronic intermittent L-DOPA administration; therefore, a reduction of L-DOPA dosage might effectively contrast the appearance of side effects (Fredduzzi et al., 2002; Grondin et al., 1999; Kanda et al., 1998; Pinna et al., 2001).

In line with these suggestions, recent preliminary clinical trials have reported that adenosine $A_{2A}$ receptor antagonists improved L-DOPA efficacy without inducing dyskinesia (Chase et al., 2003; Kase et al., 2003; LeWitt, 2004).

Adenosine $A_{2A}$ receptor antagonists are among the most promising class of drugs for the treatment of Parkinson's disease, therefore, the synthesis of new adenosine $A_{2A}$ receptor antagonists and their testing in models of Parkinson's disease are of great importance for the development of new, more effective treatments, devoid of side effects.

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