

SYNERGISTIC EFFECT OF SCH 58261, AN ADENOSINE A_{2A} RECEPTOR ANTAGONIST, AND L-DOPA ON THE RESERPINE-INDUCED MUSCLE RIGIDITY IN RATS

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Synergistic effect of SCH 58261, an adenosine A_{2A} receptor antagonist, and L-DOPA on the reserpine-induced muscle rigidity in rats. J. WARDAS, Pol. J. Pharmacol., 2003, 55, 155–164.

The aim of the present study was to find out whether a blockade of adenosine A_{2A} receptors by the selective antagonist, SCH 58261, potentiates the attenuating effect of L-DOPA, the well-known antiparkinsonian drug, on parkinsonian-like muscle rigidity in rats. Muscle tone was examined using a combined mechano- and electromyographic method, which simultaneously measured muscle resistance of a rat hindfoot to passive extension and flexion in the ankle joint and the electromyographic (EMG) activity of the antagonistic muscles of that joint: gastrocnemius and tibialis anterior. Muscle rigidity was produced by reserpine (5 mg/kg *ip*) injected in combination with α -methyl-p-tyrosine (α -MT, 250 mg/kg *ip*). L-DOPA (25 mg/kg *ip*) or SCH 58261 (0.1 mg/kg *ip*) administered separately, slightly influenced the reserpine + α -MT-induced muscle rigidity. However, only ankle joint extension was affected significantly while the effect on flexion of the rat hindfoot was not significant. Neither L-DOPA nor SCH 58261 given separately modified the reserpine-enhanced tonic or reflex EMG activities in both muscles examined. However, when L-DOPA (25 mg/kg) was given together with SCH 58261 (0.1 mg/kg), a clear synergistic effect was seen on both examined movements and muscles. The present results show that the blockade of adenosine A_{2A} receptors potentiates the antiparkinsonian effect of L-DOPA. Since such an effect was seen in different animal models of Parkinson's disease (PD), it seems that co-administration of SCH 58261 may allow for the lowering of the doses of L-DOPA in clinical practice, which indicates a potential therapeutic value of this compound in the treatment of PD.

Key words: adenosine A_{2A} receptors, L-DOPA, mechano- and electromyogram, muscle rigidity, parkinsonism, reserpine, SCH 58261

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INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of the dopaminergic neurons in the substantia nigra pars compacta which innervate the dorsal striatum. In consequence, a deficit of dopamine is seen in the striatum which leads to the development of numerous symptoms of PD, such as akinesia, muscle rigidity and tremor [2]. The most effective and commonly used drug which ameliorates these symptoms is a dopamine precursor, levodopa (L-DOPA), however, its use is associated with the occurrence of long-term complications which include progressive loss of drug efficacy and appearance of side effects such as dyskinesia, on-off phenomena or psychotic symptoms.

It is well known that besides dopamine also other neurotransmitter systems such as glutamate, GABA or acetylcholine seem to be involved in development of PD symptoms [14, 31]. Recently, it has been shown that also modulation of adenosine A_{2A} receptors in the basal ganglia structures strongly influences the motor behavior in animals [4, 7, 12, 13, 20, 24, 25, 29, 31, 37, 38]. These receptors are localized mainly in the striatum together with dopamine D₂ receptors on GABAergic medium-sized spiny neurons, which also contain enkephalin and project to the globus pallidus [8, 10, 30, 33, 35]. Studies with selective antagonists of A_{2A} receptors have shown that these compounds suppress parkinsonian-like symptoms (e.g. catalepsy, akinesia) in rodents and primate models of PD [12, 13, 24, 31, 38] and potentiate the effect of L-DOPA in rats unilaterally lesioned with 6-OHDA [4]. Therefore, it has been suggested that the blockade of adenosine A_{2A} receptors could be beneficial in the treatment of PD.

It is well known that muscle rigidity is one of the symptoms of PD, in addition to akinesia and tremor. We previously demonstrated using a mechanomyographic method that muscle rigidity induced by reserpine or haloperidol in rats [21, 23] was similar to that found in parkinsonian patients [27], since both the increased resistance of hindlimbs to passive displacement and potentiation of EMG components in response to passive movements were observed. Furthermore, similarly to parkinsonian patients, a tonic electromyographic activity was seen at rest [21, 23]. Using this method we have also shown that the haloperidol-induced

muscle rigidity is diminished by e.g. L-DOPA, pramipexole (a dopamine D₂/D₃ agonist) and by SCH 58261, a selective antagonist of adenosine A_{2A} receptors [22, 38].

The present study was aimed at determining whether blockade of adenosine A_{2A} receptors with SCH 58261 (5-amino-7-(β-phenylethyl)-2-(8-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine), is capable of potentiating the effect of low doses of L-DOPA on the parkinsonian-like muscle rigidity induced by reserpine + α-methyl-p-tyrosine (α-MT) in rats. Muscle rigidity was examined using a combined mechano- and EMG method which simultaneously measured muscle resistance (MMG) of a rat hindfoot to passive extension and flexion in the ankle joint, and the EMG activity of the antagonistic muscles of that joint, gastrocnemius and tibialis anterior.

MATERIALS and METHODS

Male Wistar rats (280–350 g) were used in all the experiments. Rats were kept in the well ventilated room on an artificial light/dark cycle (12/12 h, light on from 7 a.m. to 7 p.m.), under standard conditions (21°C) with a free access to food and water. All the experiments were carried out in compliance with the Animal Protection Bill of August 21, 1997; (published in Dziennik Ustaw no. 111/1997 item 724), and according to the NIH Guide for the Care and Use of Laboratory Animals.

Drugs

SCH 58261 (kindly donated by Schering-Plough, Milan, Italy) was suspended in a 0.5% methylcellulose (Sigma, USA) and injected at a dose of 0.1 mg/kg in a volume of 1 ml *ip*/100 g. Reserpine (Polfa, Warszawa, Poland) was dissolved in a solution containing a 0.25% citric acid, 2% benzyl alcohol and 10% Tween 80 and injected at a dose of 5 mg/kg (0.4 ml/100 g). α-MT was dissolved in distilled water and injected at a dose of 250 mg/kg (0.4 ml/100 g). L-DOPA (Sigma-Aldrich, USA) was suspended in 1% Tween and administered at a dose of 25 mg/kg *ip*. Benserazide (6.25 mg/kg *ip*, Sigma-Aldrich, USA) was dissolved in distilled water. All the solutions and suspensions were freshly prepared prior to each injection. Control rats were administered with the appropriate vehicles.

Treatment scheme

In the first group, rats were treated with reserpine (5 mg/kg *ip*) or vehicle and 16 h later with α -MT (250 mg/kg *ip*); SCH 58261 (0.1 mg/kg *ip*) or vehicle (0.5% methylcellulose) was injected 3 h 45 min after α -MT (19 h 45 min after reserpine).

The second group of rats was treated with reserpine (5 mg/kg *ip*) and 16 h later with α -MT (250 mg/kg *ip*); benserazide hydrochloride (6.25 mg/kg *ip*) was given 3 h 30 min after α -MT and 20 min later rats received L-DOPA (25 mg/kg *ip*).

The third group of rats was administered with reserpine (5 mg/kg *ip*) and 16 h later with α -MT (250 mg/kg *ip*); additionally at 3 h 30 min after α -MT rats received benserazide hydrochloride (6.25 mg/kg *ip*), 15 min later SCH 58261 (0.1 mg/kg *ip*) and 5 min later L-DOPA (25 mg/kg *ip*, 3 h 50 min after α -MT).

Mechano- and electromyographic measurements always began 4 h after α -MT (20 h after reserpine) and lasted 90 min.

Mechano- and electromyographic measurement

Simultaneous measurement of the muscle resistance of the rat's hind limb (MMG) and the EMG activity of muscles, developed in response to passive movements were described previously [21–23, 38]. Briefly, each rat was placed in a metaplex cage, well ventilated and adapted to its size. The rat's hind foot which protruded from an opening at the bottom of the cage was placed on an appropriately matched metaplex block and gently fixed to it with an adhesive tape. Two pairs of flexible stainless steel wire electrodes (Cooner Wire, Chatsworth, Calif., USA) which were teflon-insulated (e.d. 0.25 mm) except for a 4-mm uninsulated part (e.d. 0.1 mm), were inserted percutaneously into the gastrocnemius (extensor, plantar flexor) and tibialis anterior (flexor, dorsal flexor) muscles. The distance between the two electrodes of a pair in each muscle was ca. 5 mm. An earth electrode was attached to the rat's tail covered with special electrode cream.

The experiment consisted of 90 successive episodes of down-and-up movements of the block (30 s apart), which passively extended and flexed by 25° the rat's foot in the ankle joint. Each movement lasted 250 ms. The metaplex block was connected to a force sensor which recorded the resistance of the foot to passive movements (a mechanical moment, torque, MMG). The maximum resistance (maximal torque (gcm), in comparison with the

premovement value) of hind leg muscles for each down (extension) or up (flexion) movement was calculated. The recording of both MMG and EMG signals started 200 ms earlier, and was carried out for 250 ms throughout and 550 ms after the end of each passive movement. EMG and MMG signals were sampled by analog-digital converters (AD), with a frequency of 10 kHz per channel, and were fed into a PC.

The EMG activity of the gastrocnemius and tibialis anterior muscles was rectified and averaged with a time constant of 20 ms for each down-and-up movement. The EMG curves generated in that way were composed of points spaced 20 ms apart. A further analysis was carried out using the averaged EMG curves. The following parameters were estimated for each movement: 1) the mean pre-movement amplitude (EMG-baseline, resting EMG activity); 2) components computed as differences between the maximum amplitude of the averaged EMG curves at three time points after the start of a movement, and the baseline: a) EMG-A (0–20 ms); b) EMG-B (60–140 ms) c) EMG-C (220–340 ms). In order to visualize the main tendency of a time course of the EMG activity during a movement, all individual EMG curves for each group of rats were superimposed and averaged for either muscle or movement.

All the cycles disturbed by voluntary movements of an animal were discarded. The number of rats in each experimental group was between 7 and 10 ($N = 7–10$). The animals were used only once. After termination of the experiment, the rats were killed by an overdose of pentobarbital (Vetbutal, Biowet, Poland).

Statistics

A statistical analysis of maximum resistance values (torque, in gcm) was carried out using the means calculated for each experimental group of rats. First, the mean maximum resistance values were calculated every 30 min for the whole experimental session (90 min) for each rat; subsequently, mean maximum resistance values were calculated for each experimental group. Mean values of the EMG parameters were calculated for the whole experimental session (90 min) as well. Statistical significance of differences was estimated using the Kruskal-Wallis test followed, if significant, by Wilcoxon's rank-sum tests (Mann-Whitney U-test) for multiple comparisons.

RESULTS

Influence of L-DOPA and SCH 58261 on the reserpine-induced muscle resistance (MMG)

Reserpine (5 mg/kg *ip*) injected in combination with α -MT (250 mg/kg *ip*) potently increased the muscle resistance developed in response to passive extension and flexion of the rat's hindlimb at the ankle joint (Fig. 1). The observed increase in muscle tone was stronger during extension than during flexion (Fig. 1) and was visible during the whole experimental session (90 min).

L-DOPA injected at the dose of 25 mg/kg *ip* (10 min before start of the measurement) jointly with benserazide hydrochloride (6.25 mg/kg *ip*, 30 min

before the measurement) slightly decreased the reserpine-induced muscle rigidity during ankle joint extension of the rat's hindlimb, however, that effect was statistically significant only during the first 30 min of the measurement (Fig. 1). Moreover, L-DOPA did not influence the muscle rigidity induced by reserpine during the flexion of the rat's hindlimb at the ankle joint (Fig. 1).

SCH 58261 injected at the dose of 0.1 mg/kg *ip* 15 min before the start of the measurement (19 h and 45 min after reserpine) inhibited the reserpine-induced muscle resistance during extension of the rat's foot at the ankle joint (Fig. 1). The observed effect was seen in all the tested rats, and it lasted till the end of the experimental session (Fig. 1). However, SCH 58261 did not influence significantly the MMG response during flexion of the rat's hind-

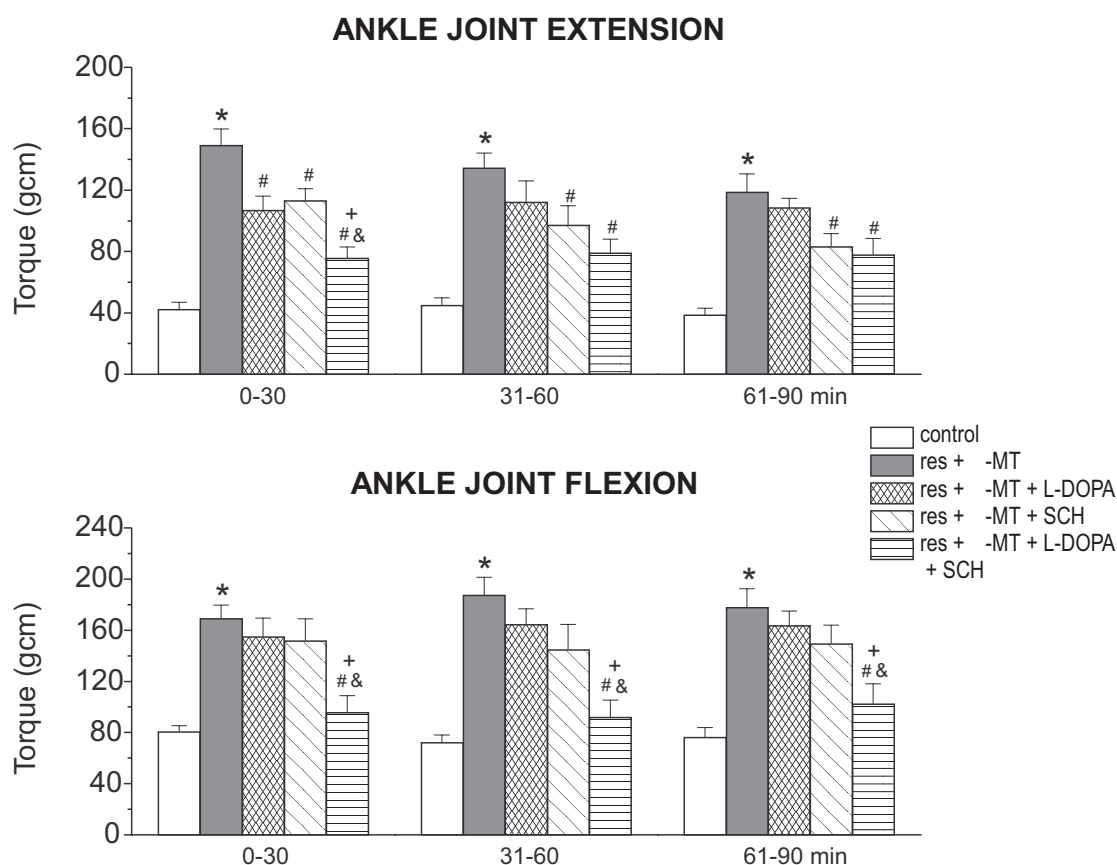


Fig. 1. The effect of combined SCH 58261 (SCH, 0.1 mg/kg) and L-DOPA (25 mg/kg + benserazide, 6.25 mg/kg) administration on the reserpine (res, 5 mg/kg + α -MT, 250 mg/kg)-enhanced muscle tone, developed during passive extension and flexion of the hind-foot in the ankle joint. Res was injected at 20 h and α -MT at 4 h before the start of the measurements. SCH 58261 was injected at 15 min, L-DOPA at 10 min and benserazide at 30 min before the start of measurements. *Abscissa*: time (in minutes) after the start of measurements; *ordinate*: maximum resistance of the hindlimb (torque in gcm). The results are shown as means \pm SEM; statistically significant differences (the Kruskal-Wallis test followed by Wilcoxon's rank-sum tests) are marked as follows: * $p < 0.05$ vs control; # $p < 0.05$ vs res; & $p < 0.05$ vs SCH 58261 0.1 mg; + $p < 0.05$ vs L-DOPA 25 mg. The number of animals in each experimental group (N) was 7–10

limb. Only some decreasing tendency was observed, but it has not reached the level of statistical significance (Fig. 1).

However, combined administration of SCH 58261 (0.1 mg/kg *ip*) and L-DOPA (25 mg/kg *ip*) diminished the reserpine-induced muscle rigidity during both movements (Fig. 1). That effect seemed to be stronger in the ankle joint flexion, since combined administration of both compounds restored the normal level of muscle tone (Fig. 1).

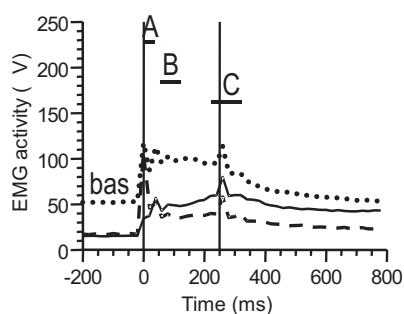
Influence of L-DOPA and SCH 58261 on the reserpine-induced electromyographic EMG activity

Tonic and reflex EMG activities are regarded as equivalents of the muscle tone, and are recorded

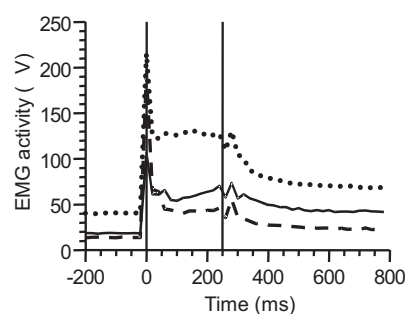
from the two antagonistic muscles: gastrocnemius and tibialis anterior before and during passive extension and flexion of the rat's hindfoot at the ankle joint. The rectified and averaged EMG activities, shown in Figure 2 A–D in the form of curves, illustrate the time-course and the intensity of EMG activities in the experimental group. The first part of each curve (between –200 and 0 ms) recorded before the start of a movement, represents tonic (resting) EMG activity (EMG baseline). Reflex EMG activity starting within 20 ms (the so called short-latency activity, EMG-A) is of spinal origin. Afterwards, the EMG activity increased gradually (EMG-B, EMG-C) until the end of movements (250 ms), after which it slowly decreased. The other part of EMG activity with a long latency is considered to be of supraspinal origin.

m. gastrocnemius

A ANKLE JOINT EXTENSION

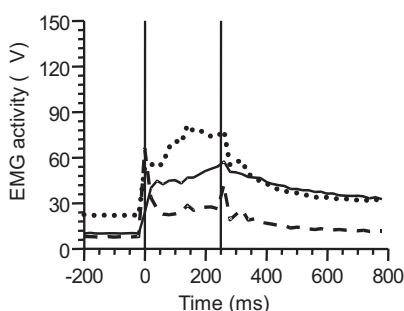


B ANKLE JOINT FLEXION



m. tibialis anterior

C ANKLE JOINT EXTENSION



D ANKLE JOINT FLEXION

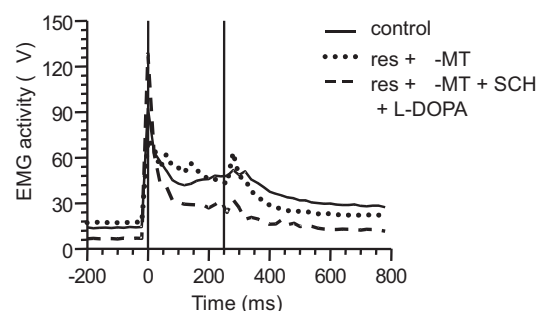


Fig. 2. The electromyographic EMG activity, recorded from the gastrocnemius (A, B) and tibialis anterior (C, D) muscles, during ankle joint extension and flexion in control rats and the animals treated with res 5 mg/kg + α -MT 250 mg/kg, and res 5 mg/kg + α -MT 250 mg/kg + SCH 58261 0.1 mg/kg + L-DOPA 25 mg/kg + benserazide 6.25 mg/kg. The EMG activity was rectified and averaged with a time constant of 20 ms. Curves were obtained by superimposing the EMG curves of all the undisturbed individual cycles, recorded for all rats in each group. The number of animals in each group (n) was 7–10. (A) Four components are shown: EMG-baseline (bas, measured before movements), EMG-A, EMG-B and EMG-C (measured between 0–20, 60–140, and 220–340 ms after the start of the movements). (A–D) Vertical lines denote the start and the end of a movement, respectively. *Abscissa*: time in ms; *ordinate*: EMG activity in μ V

Systemic injection of reserpine (5 mg/kg *ip*) given jointly with α -MT (250 mg/kg *ip*) potentially affected the EMG activity in the gastrocnemius muscle during both extension and flexion when measured at 20 h after the administration (Fig. 2A–B, 3A–B), whereas in the tibialis anterior muscle, this combination changed only slightly the physiological EMG response to movements (Fig. 2C–D, 3C–D). In the gastrocnemius muscle, reserpine caused a significant increase in the tonic EMG activity (pre-movement amplitude, EMG baseline, Fig. 2A–B, 3A–B) as well as in long-latency reflex EMG activity (EMG-B, Fig. 3A–B) in comparison with the control, solvent-treated rats. In the tibialis anterior muscle, reserpine tended to increase the tonic EMG activity (EMG baseline) at the ankle joint extension, however, this effect was not statistically significant (Fig. 2C, 3C). The only significant increase in the tibialis muscle activity was

seen in the long-latency reflex activity (EMG-B, $p < 0.05$) during ankle joint extension (Fig. 3C).

SCH 58261 given at the dose of 0.1 mg/kg *ip* did not change the reserpine-induced EMG tonic and reflex activities during either movements (Fig. 3A–D). Similarly, systemic administration of L-DOPA (25 mg/kg *ip* + benserazide hydrochloride 6.25 mg/kg *ip*) did not modify the EMG activity enhanced by reserpine (Fig. 3A–D). However, combined administration of both compounds, SCH 58261 and L-DOPA at doses which *per se* did not influence the EMG activities, strongly and significantly counteracted the reserpine-induced EMG tonic, baseline activity in both muscles and movements (Fig. 2A–D, 3A–D). Moreover, the reversal of reflex EMG activity (EMG-B) in the gastrocnemius muscle during both movements and in the tibialis muscle during ankle joint extension was seen (Fig. 2A–C, 3A–C).

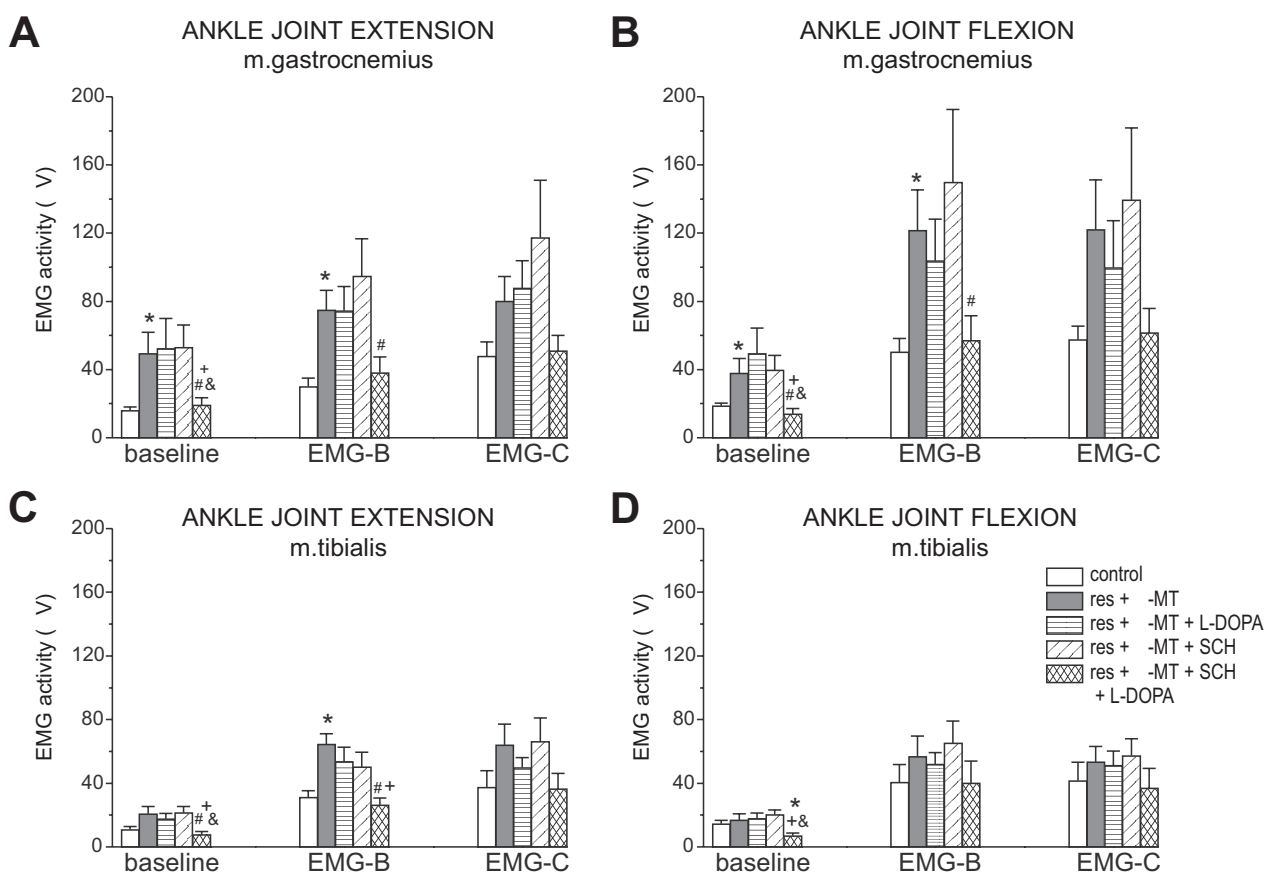


Fig. 3. A quantitative comparison of the EMG activity recorded from the gastrocnemius (A, B) and tibialis anterior (C, D) muscles during extension and flexion of the rat's hindfoot. The results are shown as means \pm SEM; statistically significant differences (the Kruskal-Wallis followed by Wilcoxon's rank-sum tests) are marked as follows: * $p < 0.05$ vs control; # $p < 0.05$ vs res; & $p < 0.05$ vs SCH 58261; + $p < 0.05$ vs L-DOPA. Ordinate: EMG activity in μ V; the number of rats in each group (n) 7–10. For further explanations, see Figure 2

The short-latency EMG reflex activity (EMG-A) after SCH 58261, L-DOPA or combined administration of both compounds showed no differences in either examined muscle and movement in comparison with the control, solvent-treated animals (data not shown).

DISCUSSION

The present results show that SCH 58261, a selective adenosine A_{2A} receptor antagonist [40], potentiates the effect of the low dose of L-DOPA in the model of reserpine-plus- α -MT-induced muscle rigidity. We have previously demonstrated that injection of some model substances which evoke parkinsonian symptoms, such as haloperidol, reserpine or bilateral 6-OHDA into the substantia nigra, evokes parkinsonian-like rigidity which develops in response to passive movements [21, 23, 39], and is characterized by: 1) increased resistance of limbs to passive displacement; 2) potentiation of EMG components, and 3) coactivation of antagonistic muscles in response to passive movements. Furthermore, similarly like in parkinsonian patients, a tonic EMG activity develops at rest, which reflects some difficulty in relaxing the muscles [19]. In the present study, the combined administration of low, subthreshold doses of SCH 58261 and L-DOPA counteracted both the muscle tone (MMG) and even stronger, the EMG activity at rest as well as the EMG long-latency reflex response to passive movements in rats previously treated with reserpine plus α -MT. Therefore, it may be concluded that such a treatment counteracted parkinsonian-like muscle rigidity and resulted in a significantly greater antiparkinsonian effect than that produced by either treatment alone.

In the present study, it was found that 2–3 times lower doses of L-DOPA were needed in order to reverse the muscle rigidity induced by reserpine plus α -MT in comparison to the haloperidol-induced muscle rigidity, studied previously [38]. The differences in doses of L-DOPA used in the present experiment vs previous one might be due to different sensitivity of dopamine receptors found in both models. It is known that reserpine evokes its behavioral effects (e.g. muscle rigidity, hypokinesia) by depleting dopamine from nigrostriatal terminals which leads to development of enhanced response to dopamine agonists that occurs rapidly following single or repeated reserpine administration [17].

These include loss of D_1/D_2 synergism or enhanced metabolic responses to D_1 agonists with increased glucose utilization in the substantia nigra pars reticulata or c-fos immunostaining [18, 32, 36]. More sensitive receptors may respond more readily to the action of dopamine agonists including L-DOPA, which when administered in low doses, similar to the dose used in the present study, is converted to dopamine also in rats injected with reserpine plus α -MT [9]. Moreover, it has been shown that blockade of adenosine A_{2A} receptors with CSC, a selective antagonist, increased the release of dopamine derived from exogenous L-DOPA both in intact and reserpinized rats [11, and personal communication] which suggest that under these conditions enhanced conversion of exogenous L-DOPA to dopamine takes place.

Furthermore, in the present study, SCH 58261 given at the same dose which was effective in the model of haloperidol-induced muscle rigidity [38], counteracted also the muscle rigidity induced by reserpine in rats. However, in both models low dose of SCH 58261 affected only ankle joint extension but not flexion of the rat's hindfoot. Moreover, since haloperidol was used at the low dose, no enhancement of the tonic and reflex EMG activity was found [38]. In contrast, in the present study, reserpine produced a strong enhancement of both muscle resistance (MMG) and tonic and reflex EMG activity. At present, no explanation can be offered for the lack of an effect of SCH 58261 administered alone on passive flexion of the rats hindfoot. However, such a relatively smaller effect on MMG during passive flexion than during extension of a rat's foot after systemic injection of even very high doses of haloperidol or after intrastriatal administration of the selective A_{2A} agonist, CGS 21680 was also seen [23, 37].

At present, it cannot be excluded that SCH 58261 may interfere with the pharmacokinetics of L-DOPA or benserazide though such an interaction has not been found as yet. However, since SCH 58261 potentiated the effect of not only L-DOPA but also selective dopamine D_1 and D_2 agonists in different animal models of PD [4, 29], it is unlikely that the observed effect could be due to a pharmacokinetic interaction.

Recently, it has been demonstrated that a blockade of adenosine A_{2A} receptors potentiates both the contralateral rotations and c-fos expression induced by L-DOPA and direct dopamine agonists in unilat-

erally 6-OHDA-lesioned rats [4, 20, 29]. However, SCH 58261 potentiated the dopamine D₁-induced increase in c-fos mRNA expression but no such interaction with dopamine D₂ agonists could be detected [20]. Therefore, it seems that the observed potentiating effect of SCH 58261 and L-DOPA on the reserpine-induced muscle rigidity might be attributed to the synergistic action of A_{2A} receptor blockade with D₁ stimulation [20, 38].

A putative molecular and cellular mechanism underlying the antiparkinsonian action of adenosine A_{2A} antagonists has been proposed to be due to the intramembrane receptor-receptor interaction between A_{2A}-D₂ in the striatum and a downstream interaction at the level of intracellular signal transduction [5, 6, 14, 20, 25, 31]. However, there is increasing evidence, that A_{2A} receptors can operate independently of D₂ receptors [1, 14–16, 31, 34]. Therefore, it seems that under normal conditions, when the dopamine nigrostriatal projection and D₂ receptor functions are preserved, both A_{2A}/D₂ intramembrane interaction as well as independent A_{2A} and D₂ receptors activities might occur in striatopallidal neurons. However, when dopamine or D₂ receptor activity is lowered or absent (e.g. in reserpine-treated animals, MPTP-treated monkeys, or D₂ knockout mice), adenosine A_{2A} receptor may still elicit its proper physiological effects independently of the antagonistic interaction with dopamine-mediated signaling [1, 14]. As already mentioned elsewhere, in PD and in animal models of this disease, depletion of dopamine triggers a cascade of functional changes including overactivation of the GABA/ENK “indirect” striopallidal pathway [10]. It has also been shown that adenosine A_{2A} receptors modulate GABAergic synaptic transmission both in the striatum and globus pallidus and inhibit GABA release from striatal synaptosomes in the absence of dopamine [14–16, 26] or in the absence of dopamine receptors in the D₂ knockout mice [1, 14]. Therefore, SCH 58261 and other selective antagonists of adenosine A_{2A} receptors may suppress the overactivity of the striopallidal pathway mainly through enhanced GABA release from presynaptic sites in the striatum, and, thus, ameliorate motor dysfunctions [14, 31]. In agreement, blockade of A_{2A} receptors decreased the extracellular level of GABA in the globus pallidus of the lesioned rats and reversed the increased expression of proenkephalin mRNA in the striatum [1, 14, 28, 31]. Additionally, further contribution to

the antiparkinsonian effect of adenosine A_{2A} receptor antagonists may be related to a cholinergic mechanism [14, 31]. It has been demonstrated that activation of A_{2A} receptors leads to enhancement of acetylcholine (ACh) release, which partly is due to reduction of the ability of dopamine D₂ receptors to inhibit the release of ACh [3, 14, 15, 31]. Thus, the blockade of adenosine A_{2A} receptors *via* inhibition of the ACh release might contribute to the release of pallidal GABA and to the antiparkinsonian properties of selective A_{2A} antagonists [14, 31].

Taken together, it may be suggested that similarly to other A_{2A} receptor antagonists, SCH 58261 by acting synergistically with L-DOPA on the muscle rigidity induced by reserpine may allow for the lowering of the doses of L-DOPA, thus providing a novel therapeutic tool for the treatment of PD.

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