Muscarinic antagonists microinjected into the subthalamic nucleus decrease muscular rigidity in reserpinized rats

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Abstract

The ability of anticholinergic agents microinjected into the subthalamic nucleus to reduce reserpine-induced muscular rigidity was assessed in rats. The electromyographical activity of the gastrocnemius-soleus muscle was used as a parameter of muscular rigidity. Reserpine (5 mg/kg i.p.) produced the appearance of electromyographical activity. The muscarinic antagonists M₃ (1.27 nmol of 4-DAMP) and M₁ (2.36 nmol of pirenzepine) markedly reduced the reserpine-induced electromyographical activity, whereas the M₂ antagonist AF-DX-116 (2.37 nmol) had no effect. These results suggest that a high cholinergic tone in the subthalamic nucleus is associated with the reserpine-induced muscular rigidity. Moreover, the M₃ muscarinic antagonist is more effective than the M₁ muscarinic antagonist in reducing the muscular rigidity in reserpinized rats, a model of Parkinson's disease, by blocking the high cholinergic tone in the subthalamic nucleus.

Keywords: Anticholinergics; Basal ganglia; Carbachol; Cholinergic action; 4-Diphenyl acetoxy-N-methyl piperidine methobromide (4-DAMP); Parkinsonism; Pirenzepine; Subthalamic nucleus

The cholinergic projection of the pedunculopontine tegmental nucleus appears to regulate the spontaneous activity of subthalamic neurons [3,5,12-14,17]. It has been shown previously that the cholinergic effect on subthalamic neurons is excitatory [7,9] and is exerted through M₃ muscarinic receptors [9]. Studies focusing on mechanisms of Parkinson’s disease have suggested that the dopaminergic innervation to the subthalamic nucleus (STN) inhibits subthalamic neurons [4,8], thus opposing the action of the cholinergic innervation. For instance, the loss of dopaminergic innervation to the striatum and to the subthalamic nucleus, induced either by neurotoxic lesion [2,4,8] or by pharmacological treatment like reserpine [10,11], produces the appearance of electromyographical (EMG) activity that could be the consequence of a high cholinergic tone in the STN. Therefore, it is reasonable to assume that the high cholinergic tone is mediating, in part, the high firing rate of subthalamic neurons occurring in parkinsonism [2]. That being the case, anticholinergic agents could control the high activity of the subthalamic neurons. In view of this possibility, we have here explored whether muscarinic antagonists microinjected directly into the STN control the muscular rigidity present in the reserpinized rat, a pharmacological model of Parkinson’s disease.

Male rats (Wistar strain, 280–300 g) bred in our facilities were used throughout the experiment. Animals were maintained under constant room temperature (23°C) and 12:12 h light/dark cycle, with food and water ad libitum. All efforts were made to minimize animal suffering and to reduce the number of animals used, as outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institutes of Health (NIH publication no. 86-23, revised 1985).

Rats were anesthetized with chloral hydrate (350 mg/kg, i.p.) and placed in a stereotaxic instrument (David Kopf) with the incisor bar 3.3 mm below the interaural line [15].

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Each rat was implanted with a guide cannula (23-gauge needle tubing); the tip was positioned 2 mm dorsal to the right STN. The coordinates were AP -3.9 mm from bregma, L -2.1 mm from the midline and P -5.3 mm from the cortex surface [15]. Guide cannulae were fixed to the skull with dental acrylic held to stainless steel screws. Stainless steel stylets were inserted into the cannula to prevent lumen occlusion. After surgery, all animals were injected with benzathine penicillin (300 000 IU/kg, i.m.) to prevent infection. The experiments were performed 7 days after surgery.

Locomotor activity was assessed in activity boxes provided with 8-infrared photocells connected to a counter. The locomotor activity of each animal was measured at 60 min before and 120 min after the reserpine (5 mg/kg, i.p.) injection. EMG activity was recorded in rats placed individually in a Plexiglass box with slots on its floor through which the hindlimbs of the rat were allowed to hang [10]. The EMG of the gastrocnemius-soleus muscle of the right hindlimb was recorded in the non-anesthetized animal for 180 min after the reserpine injection, using pairs of platinum needle electrodes (Gras Inst., Quincy, MA) inserted percutaneously into the muscle. Electrical signals were bandpass filtered (100 Hz to 10 kHz) and amplified before being stored in audio tapes for off line analysis. A 30-gauge stainless steel infusion cannula with conic tip was connected via PE10 tubing to a 2 µl microsyringe (Hamilton). After loading with the vehicle or drug solution, the infusion cannula was slowly introduced into the guide cannula. Once inserted, the infusion cannula protruded 2 mm from the tip of the guide cannula. Vehicle or drug solution were manually injected into the STN in a volume of 0.25 µl over 5 min (0.05 µl/min). After completion of the injection, the cannula was left in place for an additional 5 min before being withdrawn. EMG activity was recorded under three testing conditions: (1) basal condition (continuous recording during 30 min), (2) after applying the vehicle (30 min recording), and (3) after applying the muscarinic antagonist (120 min recording). After sorting the signals by their amplitude (window discrimination), frequency histograms of the motor units were obtained using a computer program [18]. Increments or decrements of the EMG activity were expressed as percentage of the basal value. Values are expressed as means ± SEM. After testing for normality of these data by Snedecor’s F-test, one-way ANOVA followed of Dunnet’s post-test were used to determine the significance of the drug effect. Significant differences were taken at a P < 0.05 level.

At the end of the experiment, rats were deeply anesthetized and perfused through the ascending aorta with 200 ml of phosphate buffered solution (PBS; pH 7.4) followed by 200 ml of 4% formaldehyde in PBS. The brain was removed and stored in the fixation solution for at least 3 days before slicing. The position of the injection sites was verified in 60 µm parasagittal sections made with a vibrotome (Oxford) and stained with Cresyl violet.
respectively) to produce the same effect. Nevertheless, the M3 antagonist was more effective than the MI antagonist, since the M3 antagonist required half of the dose of MI (1.27 nmol/0.25 µl against 2.36 nmol/0.25 µl, n = 5) into the STN markedly reduced the EMG activity (Fig. 2). The effect was still present 150 min after the administration of the antagonists (Fig. 2). In contrast, the microinjection of the M2 muscarinic antagonist AFDX-116 (2.36 nmol/0.25 µl, n = 5) caused no significant change in the EMG activity (Fig. 2). The microinjection of 0.25 µl of the vehicle did not affect the EMG activity in any of the rats (Fig. 2).

These results suggest that reserpine may enhance the activity of subthalamic neurons as reflected by the appearance of EMG activity in the gastrocnemius-soleus muscle that has been considered a sign of muscular rigidity [8,10,11]. The reserpine-induced muscular rigidity found here is consistent with previous studies [10,11]. Moreover, our results show that both M1 (pirenzepine) and M3 (4-DAMP) muscarinic antagonists microinjected into the STN decrease the muscular rigidity of the reserpinized rat, as revealed by the decrease in the EMG activity. However, the M3 antagonist was more effective than the M1 antagonist, since the M3 antagonist required half of the dose of M1 (1.27 nmol/0.25 µl against 2.36 nmol/0.25 µl, respectively) to produce the same effect.

In experimental Parkinsonism, either in monkeys [2,11] or in rats [8,10], muscular rigidity appears to be the consequence of an increased nigrofugal output as result of an increased excitatory input from STN to substantia nigra pars reticulata (SNr) [16]. The increased activity of STN neurons may, in part, be due to a disinhibition from the pallidal input [1,6] and the loss of inhibitory action of dopamine innervation from the substantia nigra pars compacta [4,8]. The present results suggest that, in part, the high firing rate of subthalamic neurons present in parkinsonism may also be due to an excitatory action of cholinergic input to the STN from the pedunculopontine tegmental nucleus. We have found recently that the cholinergic excitation of subthalamic neurons is mediated by M3 muscarinic receptors [9]. Therefore, this finding, taken together with the present results, raises the possibility that, in Parkinson’s disease, the excessive firing of subthalamic neurons [2] could be controlled more selectively by M3 muscarinic antagonists than by M1 muscarinic antagonists.

Under physiological conditions, the cholinergic input from the pedunculopontine tegmental nucleus to the STN [3,5,12-14] may exert an excitatory regulation on subthalamic neurons through M3 muscarinic receptors [7,9]. The subthalamic neurons may also exert an excitatory regulation on SNr and entopeduncular (EPN) neurons through a glutamatergic innervation [16]. Therefore, it can be expected that blocking the cholinergic tone in the STN with muscarinic M3 antagonist (such as 4-DAMP) would decrease the firing rate of subthalamic neurons [9] and consequently that of SNr/EPN neurons. The decrement of the inhibitory output from SNr/EPN to thalamus would enhance the facilitatory input from thalamus to the motor cortex [1,6]. The result would be an increased motor output from cortex to lower motor centers [2,6] that would increase the motor activity or would decrease the muscular rigidity [1,6].

In summary, the present results suggest that the STN may be another target of anticholinergic agents in the control of parkinsonism.

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