M3 muscarinic receptors mediate cholinergic excitation of the spontaneous activity of subthalamic neurons in the rat

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Abstract

The effect of the muscarinic antagonist on carbachol-induced increase in spontaneous activity of neurons of the subthalamic nucleus was examined by recording the extracellular unitary activity in an in vitro slice preparation. Carbachol produced (98% of the 263 neurons tested) an increase (twofold of the basal at 500 nM) of the discharge frequency. The EC50 for the carbachol-induced effect was $375 \pm 8.7 \text{nM}$ (mean $\pm$ SEM). The response was blocked by muscarinic antagonists in a dose dependent manner. However, the IC50 (94 $\pm$ 3 nM) for the M3 antagonist 4-diphenyl acetox-y-N-methyl piperidine methobromide (4-DAMP) was considerably less than the other muscarinic antagonists (M1 antagonist pirenzepine, IC50 1340 $\pm$ 110 nM; M2 antagonist AF-DX-116, IC50 6780 $\pm$ 690 nM). These results suggest that the cholinergic input to the rat subthalamic nucleus exerts a postsynaptic excitatory action and this effect is likely mediated via muscarinic receptor type 3.

Keywords: Basal ganglia; Subthalamic nucleus; Carbachol; Cholinergic action; 4-Diphenyl acetox-y-N-methyl piperidine methobromide; Pirenzepine; Trihexyphenidyl

The pedunculopontine tegmental nucleus appears to regulate the spontaneous activity of subthalamic neurons through a cholinergic projection [8,14,15,16,18]. Electrophysiological studies [12] have shown that acetylcholine excites the subthalamic neurons, but, so far, the type of cholinergic receptor that mediates the effect is unknown. To address this issue, we have examined the effect of a specific cholinergic antagonist on the excitation produced by carbachol, a non-selective agonist, on the spontaneous activity of subthalamic neurons recorded in an in vitro slice preparation.

Adult male Wistar rats, weighing 190–210 g, were anesthetized with chloral hydrate (300 mg/kg i.p.) and perfused intracardiacally with 50 ml of cold (4°C) sucrose artificial cerebrospinal fluid (sucrose-ACSF) [1,13]. Following decapitation, the brain was rapidly removed from the skull and immersed in cold sucrose-ACSF. Sagittal slices (400 μm thick) were obtained using a vibroslicer. The slices were maintained in oxygenated (5% CO2 in O2) sucrose medium for 60 min, and then transferred to an immersion recording chamber and superfused with oxygenated artificial cerebrospinal fluid. The composition of ACSF was (mM): NaCl, 126; KCl, 5; CaCl2, 2; MgSO4, 2; NaHCO3, 26; NaH2PO4, 1.25; d-glucose, 10. For the sucrose-ACSF, NaCl was equimolarily substituted by sucrose. To obtain a calcium-free, high magnesium ACSF, CaCl2 was removed and MgSO4 content was increased to 9 mM [13]. Extracellular unitary activity was recorded by using glass microelectrodes filled with 2 M NaCl (10–12 MΩ). Signals were processed and analyzed using an on-line spike data acquisition program [19].

Carbachol, mecamylamine and nicotine were obtained from Sigma (St. Louis, MO, USA). Pirenzepine and 4-diphenyl acetox-y-N-methyl piperidine methobromide (4-DAMP) were purchased from RBI (Natick, MA). Trihexyphenidyl was a gift from Cyanamid of Mexico and AF-DX-116 was a gift from Dr. Karl Thomae (Biberach an der Riss, Germany).

Two hundred sixty-three subthalamic neurons from 83 rats were studied. All neurons tested displayed spontane-
The increase in firing rate induced by carbachol was concentration-dependent (0.1–10 μM) (Fig. 1a). The minimum concentration to see the effect was 0.1 μM and the maximum was observed at 10 μM. The EC50 for the increase in firing rate was 375 ± 8.7 nM. The repetitive administration of carbachol did not diminish its effect (Fig. 1b). The carbachol effect was dose-dependently blocked (IC50 94 ± 3 nM) by the M3 antagonist 4-DAMP (Figs. 1b and 2). As can be seen in Fig. 1B, the blockade of carbachol by 4-DAMP persisted after the withdrawal of the compound from the superfusion medium. Pirenzepine (M1 antagonist), also blocked (IC50 1340 ± 110 nM) the carbachol (500 nM) response (Fig. 2). The M1 antagonist trihexyphenidyl, also blocked the carbachol-response at the same concentration of pirenzepine (not shown). The carbachol response was also blocked (IC50 6780 ± 690 nM) by the M2 antagonist AF-DX-116 (Fig. 2). In contrast to muscarinic antagonists, the antagonist of CNS nicotinic receptors mecamylamine (10 and 100 μM, n = 5) did not block the response to 500 nM carbachol. In line with this, nicotine (10 and 100 μM, n = 4) had no effect on the spontaneous firing of subthalamic neurons. To differentiate presynaptic from postsynaptic effects, we tested the action of carbachol in a free calcium, high magnesium medium. The discharge rate of the subthalamic neurons decreased following 15 min in this medium (17.8 ± 3.2 Hz to 5.7 ± 1.3 Hz), 500 nM carbachol still increased (to 12.2 ± 2.6 Hz; 214% increase, n = 5) the discharge frequency.

These results show that carbachol mainly enhances the activity of subthalamic neurons and suggest that under physiological conditions the cholinergic input from the pedunculopontine tegmental nucleus [8,14,15,16,18], may exert an excitatory command on subthalamic neu-

Fig. 1. (A) Concentration-response curve of the effect of carbachol on the spontaneous activity of subthalamic neurons. Each concentration was tested on nine different cells. (B) Blockade of the effect of carbachol (500 nM) by two concentrations (100 and 500 nM) of 4-DAMP (added 3 min before carbachol) and recovery of carbachol effect.

Fig. 2. Concentration-inhibition curves of muscarinic antagonists on the effect of carbachol on the spontaneous activity of subthalamic neurons. The response to 500 nM carbachol was measured first alone and, after recovery of basal activity, in the presence of the antagonist. Carbachol was presented in the medium during 3 min. Antagonists were added 3 min before carbachol. Separate slices were used to test each concentration of the antagonist (each concentration was tested only once). Antagonism was evaluated expressing the response in the presence of the antagonist as a percentage of the response to carbachol alone. Each point is the mean ± SEM of 6–8 determinations.
rons through a M₃ receptor-mediated effect, as indicated by low concentration of 4-DAMP used to blockade the carbachol-effect. The carbachol-induced excitation of subthalamic neurons found here is consistent with previous work [12].

Consistent with previous reports [12] the effect of carbachol was still present in a calcium-free, high magnesium medium, a condition that prevents synaptic medium effects. These results suggest that the carbachol action may be at the postsynaptic level.

The heterogeneous nature of the muscarinic receptors has been demonstrated with cloning of five different muscarinic receptors designated m₁, m₂, m₃, m₄ and m₅ [6,7]. However, conventional pharmacological studies have only identified at least three subtypes of muscarinic receptors subclassified as M₁, M₂ and M₃ [5,10,20,21]. The present results show that, although antagonists of all these three subtypes of receptors can block the carbachol-induced excitation of subthalamic neurons, the M₃ antagonist 4-DAMP blocked the carbachol effect at lower concentration. Previous work [3,10] has demonstrated that 4-DAMP exhibits high affinity for the M₃ receptor whereas its affinity for M₁ and M₂ receptors is significantly lower [10]. Accordingly, 4-DAMP in low concentrations is a useful ligand to label M₃ receptors sites in certain regions of the rat brain that are enriched with this subtype of receptors [3]. The low concentrations of 4-DAMP, compared with those of pirenzepine and AF-DX-116, required to block the carbachol-response suggest that the carbachol effect was mediated by an M₃ muscarinic receptor. This raises the possibility that, under physiological conditions, these receptors could mediate the cholinergic action on the spontaneous activity of subthalamic neurons.

As indicated by the failure of mecamylamine to block the carbachol effect and by the lack of effect of nicotine, nicotinic receptors do not appear to be involved in the cholinergic action in the subthalamic nucleus. These results are consistent with those of Feger et al. [12], who also did not find any effect of nicotine on the activity of subthalamic neurons.

The subthalamic neurons send an excitatory glutamatergic [17] projection to substantia nigra pars reticulata (SNr) and entopeduncular nucleus (internal segment of globus pallidus in primates). Therefore, a blockade of the cholinergic input to the subthalamic nucleus by anticholinergics, such as trihexyphenidyl, used to control Parkinsonism in clinics, would result in a decrease in the firing rate of the subthalamic neurons, and consequently in decrease of neural firing in these output nuclei of the basal ganglia. In experimental Parkinson’s disease, Parkinsonian symptoms are drastically reduced by the lesion of the subthalamic nucleus [4]. Amelioration of Parkinsonism after the lesion appears to be a consequence of a reduced inhibitory output from the basal ganglia [2,9]. Therefore, an additional target of anticholinergic agents to control Parkinsonism could be the subthalamic nucleus. As indicated by the present results, M₃ antagonists would be the best agents to control in part the excessive firing of subthalamic neurons assumed to be present in Parkinson’s disease. In line with this idea, 4-DAMP stereotactically injected into the subthalamic nucleus markedly reduced (unpublished results) muscular rigidity present in reserpine-treated (Parkinsonian) rats.

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[14] Nomura, S., Mizumo, N. and Sugimoto, T., Direct projection from


