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Research Report

Muscarinic modulation of synaptic transmission in slices of the rat ventral striatum is dependent on the frequency of afferent stimulation

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Abstract

Extracellular, intracellular and tight-seal patch-clamp recordings in ventral striatal slices were used to investigate whether the effectiveness of muscarinic neuromodulation of fast synaptic transmission may be dependent on the frequency of afferent stimulation. In all neurons tested, EPSPs were reversibly attenuated by muscarine or carbachol. This action was completely antagonized by atropine or pirenzepine. Several observations indicated a presynaptic site of action. In extracellular recordings, carbachol reduced the monosynaptic population spike but not the non-synaptic compound action potential. The acetylcholinesterase inhibitors eserine and pyridostigmine also induced an atropine-sensitive reduction of the EPSP. When the rate of afferent stimulation was increased, control EPSPs or EPSCs exhibited a decline in peak amplitude until reaching a steady-state value. Muscarinic modulation of steady-state EPSPs/EPSCs was significantly stronger in the range of lower frequencies (0.25–4 Hz) than at higher frequencies (8 and 12 Hz). The GABA_A and GABA_B-receptor/channel antagonists picrotoxin and 2-hydroxy-saclofen, the opiate receptor antagonist naloxone and atropine failed to alter the shape of the frequency–response curve. These results show that both exogenous and endogenous muscarinic receptor agonists are capable of activating a presynaptic mechanism by which fast excitatory inputs to the ventral striatum are depressed. The depressive effect is clearly stronger at lower rates of afferent stimulation than at high rates. This frequency-dependent attenuation of excitatory synaptic inputs exemplifies a new type of activity-dependent neuromodulation in central neural circuits.

Key words: Acetylcholine; Frequency depression; Glutamatergic synapse; Neuromodulation; Nucleus accumbens; Presynaptic; Rhythmicity; Stimulus rate

1. Introduction

It is well known that the effectiveness of neuromodulators may vary depending on the state of the neural network in which they operate. Many examples have been found of voltage-dependent actions of catecholamines, neuropeptides and other modulatory substances on postsynaptic target sites. Of equal importance are those cases where neuromodulatory actions are contingent upon the presence of extra- or intracellular messengers or cofactors. Surprisingly few physiological studies in the central nervous system (CNS) have been devoted to studying whether the effectiveness of neuromodulators is conditional upon the tem-

poral structure of afferent inputs to the target structure. In this study, we investigated whether the depressive effect of muscarinic receptors on limbic inputs to the ventral striatum (mainly consisting of the nucleus accumbens) depends on the frequency of stimulation of these inputs.

We considered this projection system attractive to study for several reasons. Firstly, multiple areas of the limbic cortex, which emit heavy glutamatergic projections to the ventral striatum [28,30], are characterized by specific rhythmic EEG activities that have been linked to the motivational and behavioural state of the animal [18,20,21,37]. Since the frequency components found in the EEG are reflected in the temporal firing patterns along the projection fibers of pyramidal neurons throughout the limbic cortex, the ventral striatum will receive glutamatergic inputs at different intervals. Secondly, an electrophysiological study of cholinergic

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action in the ventral striatum may help to understand the effects of cholinergic agonists on behaviour. Following injection of the cholinergic agonist carbachol into the ventral striatum an increased spontaneous locomotor activity [3] and an enhancement of dopamine-induced locomotion [17] were observed. Thirdly, the cholinergic system of the dorsal and ventral striatum has been implicated in both neurological and psychiatric disorders, e.g., Alzheimer's disease [19] and Parkinson's disease [4,9,33].

Previously, muscarinic attenuation of fast synaptic transmission has been reported for a number of brain areas, viz. hippocampus [36], striatum [1,22,34], amygdala [34,39] and neocortex [24,38]. It is quite likely that our findings on frequency-dependent modulation in the ventral striatum can be extrapolated to other brain areas showing this modulation, provided that the mechanism of action is uniform across these structures. Except for the observation that muscarine does not affect glutamate-induced depolarizations in the dorsal and/or ventral striatum and amygdala [34], no specific information is available on the mechanism of synaptic cholinergic modulation in the nucleus accumbens. Therefore we considered it useful to conduct a series of experiments elucidating this mechanism. First, we will present findings on the pre- or postsynaptic localization of muscarine action and on the modulatory effects of endogenous acetylcholine (ACh) in the ventral striatum. As for the issue of rhythmicity, we examined whether muscarinic receptors differentially modulate synaptic responses elicited at various stimulus rates, impairing transmission of inputs at some frequencies but not at others. These stimulus rates were chosen so as to reflect patterned activity in the range of rhythmic slow activity (4–12 Hz) [21] as well as low-frequency, singular inputs.

2. Materials and methods

2.1. Preparation, recording and stimulation

Eighty-six slices were obtained from male Wistar rats (80–160 g) killed by decapitation. Parasagittal slices (400 μ m; see [30]) were cut in ice-chilled artificial cerebrospinal fluid (ACSF) using a vibroslice (Campden, UK). After cutting, the slices were stored in ACSF, kept at room temperature and allowed to recover for at least 1 h. Subsequently, one of the slices was transferred to the recording chamber, completely submerged, fixed between a nylon and a plastic net and superfused (1.5–2.5 ml/min) with oxygenated (95% O₂/5% CO₂) ACSF (32–34°C, pH 7.3; composition in mM: NaCl 122.0, KCl 3.5, MgSO₄ 1.3, CaCl₂ 2.5, NaH₂PO₄ 1.0, NaHCO₃ 26.2, D-glucose 10.0).

Bipolar, biphasic rectangular current pulses of 0.2 ms duration were applied to the slice through two 60 μ m thick stainless steel electrodes, insulated except at the tip and separated by 100–200 μ m. The stimulus rate was 0.1 Hz unless noted otherwise. The stimulus intensity (range 40–120 μ A) was adjusted to evoke 60–80% of the EPSP amplitude just below spike threshold or a half-maximal popu-

lation spike in extracellular recordings. Stimulus electrodes were placed at the border of the infralimbic cortex and the rostral part of the ventral striatum ('subcortical stimulation'; see [25]), in the subicular afferents to the Acb ('fornix stimulation') or in the neuropil of the nucleus itself ('local stimulation'). Stimulation and recording sites were distributed across the shell and core of the ventral striatum at many positions along the anterior-posterior axis. The recording electrode was positioned within a distance of 200–800 μ m from the stimulus electrode.

Microelectrodes used for current clamp recordings were glass pipettes filled with 4 M potassium acetate (80–120 M Ω ; pH 7.4). Field potentials were recorded using glass pipettes containing 3 M NaCl (4–10 M Ω). Intra- and extracellular recordings were amplified by an Axoclamp 2A (Axon Instruments, USA) and relayed to a MacLab System (World Precision Instruments, USA) for averaging, storage and off-line analysis. The DC membrane potential was continuously monitored on a chart recorder. The traces shown in the figures represent averages of four sweeps. EPSPs, EPSCs and voltage responses to intracellularly injected current were sampled at 10 kHz, whereas field potentials were sampled at 12–20 kHz. Cells were accepted for experimentation when their action potential amplitude was at least 80 mV (quantified from baseline), their input resistance exceeded 15 M Ω (assessed by +0.2 nA current pulses of 50–70 ms duration) and their resting membrane potential was more negative than –70 mV.

We adopted the 'blind' in situ patch-clamp technique [5,14] for making whole-cell voltage-clamp recordings. The glass pipettes (5–8 M Ω) contained (in mM): KF 140, NaCl 5, Hepes 10, EGTA 10, MgATP 2, MgCl₂ 2, CaCl₂ 1, GTP 0.3. The osmolarity was adjusted to 275–285 mOsm and the pH to 7.3–7.4. Rectangular voltage commands (0.2 mV, 100 Hz, generated by an Axoclamp 1D amplifier, Axon Instruments, USA) were used to detect the close vicinity of neuronal membranes. Giga-seals (1–6 G Ω) were formed by applying negative pressure to a syringe connected to the electrode holder. Membrane rupture was established by slight mouth suction. The input impedance of the recorded neurons was 217 ± 33 M Ω ($N = 6$; mean $\pm$ S.E.M.). EPSCs were evoked as described above and filtered at 2 kHz. The holding potential was set at –80 mV.

2.2. Drugs and quantification

Drugs used in this study were: atropine sulphate, carbachol, eserine (physostigmine), glycine, muscarine chloride, pirenzepine (Sigma, USA), L-glutamate, 2-hydroxy-saclofen, 6-cyano-7-nitro-quinoline-2,3-dione (CNQX; Tocris Neuramin, UK), pyridostigmine (Hoffmann-La Roche, Switzerland). All of these substances were administered through the perfusion system.

A normalized value for the EPSP peak amplitude under drug conditions was obtained by averaging the peak amplitude of eight individual test EPSPs and by subsequently dividing this mean value by the average obtained in the control period. All neuronal response parameters mentioned below are expressed as mean $\pm$ S.E.M. Wilcoxon's matched-pairs signed-rank test was used for statistical evaluation of the results.

3. Results

3.1. Presynaptic modulation by muscarinic receptors

Intracellularly recorded neurons generally exhibited the characteristics of medium spiny neurons, i.e., inward rectification [8,26,35], relatively negative resting membrane potentials (-81 ± 1 mV; $N = 59$), input re-

sistances of $42 \pm 2 \text{ M}\Omega$ and action potentials $94 \pm 1 \text{ mV}$ in amplitude. Furthermore, spike trains exhibited a modest frequency adaptation.

Subcortical, fornix or local stimulation elicited depolarizing postsynaptic potentials consisting of an α -amino - 3-hydroxy-5-methyl - 4-isoxazolepropionic acid (AMPA)/kainate receptor-mediated EPSP and a slightly delayed GABA_A receptor-mediated IPSP [26,30]. The muscarinic receptor-mediated effects described here primarily involve the EPSP component of the synaptic response, since the IPSP component contributes only little to the depolarizing response evoked at resting membrane potential [30]. In all cells tested, the cholinergic agonist carbachol reversibly attenuated these responses. This effect was dose-dependent. During application of $1 \mu\text{M}$ carbachol, EPSPs were reduced from $12 \pm 1 \text{ mV}$ to $72 \pm 9\%$ of control ($P < 0.05$; washout: $89 \pm 6\%$; $N = 6$). At $10 \mu\text{M}$ carbachol, EPSPs of $17 \pm 2 \text{ mV}$ under control conditions decreased to $58 \pm 3\%$ (Fig. 1C–E; $P < 0.001$; washout: $96 \pm 3\%$ of control; $N = 19$). The effect induced by $100 \mu\text{M}$ carbachol was of a similar strength (control: $15 \pm 2 \text{ mV}$; drug: $54 \pm 3\%$; $P < 0.01$; washout: $91 \pm 3\%$ of control, $N = 12$). The attenuation of the EPSP remained intact when adding the GABA_A -receptor coupled Cl^- channel blocker picrotoxin ($10 \mu\text{M}$) to the ACSF. In comparing the effects of $10 \mu\text{M}$ carbachol in the core and shell of the ventral striatum, no significant difference in modulation strength was found. Furthermore, the degree of attenuation was similar for responses evoked by local, fornix or subcortical stimulation. Carbachol reduced EPSPs to a similar extent across a large range of the input–output curve, from intensities just evoking an EPSP to spike threshold levels. Carbachol effects were fully antagonized by the muscarinic receptor antagonists atropine ($1 \mu\text{M}$; $N = 6$) and pirenzepine ($1 \mu\text{M}$; $N = 5$; not shown). Muscarine ($10 \mu\text{M}$; $N = 8$) depressed synaptic responses to a similar extent as carbachol.

We found three indications for a presynaptic site of muscarinic modulation in the ventral striatum. First, the passive membrane properties did not change significantly during carbachol ($10 \mu\text{M}$) application. The resting membrane potential under carbachol ($10 \mu\text{M}$) conditions was $-81 \pm 2 \text{ mV}$, as compared to $-82 \pm 2 \text{ mV}$ both in control and after washout ($N = 18$). Furthermore, we quantified the steady-state amplitude of electrotonic voltage responses to intracellularly injected current pulses (0.2 – 0.6 nA positive current; duration 40 – 70 ms , applied about 50 ms after synaptic stimulation; Fig. 1A). The mean input resistance, calculated from these voltage responses, was slightly but insignificantly enhanced during muscarine ($10 \mu\text{M}$; Fig. 1A; control: $49 \pm 6 \text{ M}\Omega$; drug: $108 \pm 4\%$; washout: $105 \pm 7\%$ of control; $N = 9$). In contrast, there was a clear reduction of the recorded EPSP (Fig. 1A; control:

Fig. 1. Presynaptic modulation of EPSPs by cholinergic agonists. A: synaptic stimulation was followed by a current pulse (0.3 nA) injected into the recorded cell. Overlaid traces show synaptic and electrotonic responses under control (large EPSP) and muscarine ($10 \mu\text{M}$, small EPSP) conditions. The electrotonic voltage response to the intracellular current pulse was not altered during muscarine application, indicating the absence of a postsynaptic shunting effect. B: intracellularly recorded depolarizations were evoked by bath-application of 2.0 mM L-glutamate (15 s at a pump speed of 4 ml/min , indicated by the three black squares under the voltage trace). The amplitude of these glutamate responses was not reduced during application of $10 \mu\text{M}$ carbachol (white horizontal bar below voltage trace). The short-lasting upward deflections in between the glutamate responses represent truncated EPSPs and associated stimulus artifacts; the sample rate was 20 Hz . It should be noted that the glutamate concentration reaching the recorded neuron was most likely considerably lower than 2.0 mM because of the short application time and partial penetration through the slice. C–E: in the same cell as in B, $10 \mu\text{M}$ carbachol reversibly attenuated the EPSP. Calibration: 6 mV , 23 ms for A; 15 mV , 4.1 min for B; 20 mV , 20 ms for C–E.

$19 \pm 2 \text{ mV}$; drug: $42 \pm 4\%$; $P < 0.01$; washout: $91 \pm 4\%$ of control). Second, changes in shape or time-course of EPSPs may be indicative of a postsynaptic modulation. Carbachol ($10 \mu\text{M}$) induced no significant alterations in the 10 – 90% rise time of the EPSP (control: 2.9 ± 0.2

ms; carbachol: $106 \pm 4\%$; washout: $103 \pm 4\%$ of control; $N = 18$) or its half-width (control 14.5 ± 0.8 ms; carbachol: $96 \pm 4\%$; washout: $96 \pm 4\%$ of control). Third, carbachol effects on both EPSPs and depolarizations evoked by bath-applied L-glutamate (1.0 or 2.0 mM applied for 15 s) were examined. Under carbachol conditions, the peak amplitude of the glutamate response remained unaltered (Fig. 1B; control: 13 ± 4 mV; drug: $106 \pm 7\%$; washout: $101 \pm 5\%$ of control; $N = 7$), whereas a clear attenuation of the EPSP occurred in the same group of cells (control: 13 ± 2 mV; carbachol: $59 \pm 4\%$, $P < 0.01$; washout: $98 \pm 7\%$). The glutamate responses were largely suppressed by the AMPA/kainate receptor antagonist CNQX ($10 \mu\text{M}$) applied in conjunction with $30 \mu\text{M}$ glycine. Previously, we have shown that CNQX blocks EPSPs in ventral striatal slices [26,30]. Furthermore, glutamate-evoked depolarizations were still present in Ca^{2+} -free bathing medium, indicating a direct action of L-glutamate on the recorded neuron.

3.2. Extracellular recordings

The intracellular recordings were complemented with a field potential study, which offers the possibility to examine both a monosynaptic population spike and a presynaptic compound action potential evoked by afferent stimulation (Fig. 2). When fornix or subcortical stimulation is used, this compound action potential reflects action potentials along afferent fibers (cf. [27]). During carbachol application, the population spike

Fig. 2. Effect of carbachol on the extracellularly recorded synaptic response. A: example of a field potential, with the compound action potential indicated by 'CAP' and the population spike by 'PS'. Upward is positive in all traces. B–C: at a dose of $10 \mu\text{M}$, carbachol reversibly attenuated the population spike, but did not affect the compound action potential. Calibration: 0.5 mV, 5 ms.

Fig. 3. Effects of the acetylcholinesterase inhibitor eserine on the intracellularly recorded synaptic response. A–C: eserine ($50 \mu\text{M}$) strongly attenuated the EPSP and this effect was fully antagonized by $1 \mu\text{M}$ atropine. D: after washout, no further recovery of the EPSP was observed.

strongly decreased in amplitude (control: 0.56 ± 0.05 mV; drug: $56 \pm 7\%$, $P < 0.01$; washout: $102 \pm 4\%$ of control; $N = 11$). In contrast, the peak amplitude of the compound action potential did not change (control: 0.49 ± 0.09 mV; drug: $101 \pm 3\%$; washout: $102 \pm 4\%$ of control).

3.3. Action of endogenous acetylcholine

In ten intra- and extracellular experiments, we investigated whether endogenous ACh is capable of modulating synaptic responses to a similar extent as the externally applied agonists carbachol and muscarine. It was noted previously that application of atropine does not significantly enhance the amplitude of the monosynaptic population spike [27], indicating the absence of a clear synaptic modulation by stimulus-evoked or spontaneously released endogenous ACh under normal conditions. We now examined whether modulatory effects of endogenous ACh can be revealed by applying the acetylcholinesterase (AChE) inhibitors eserine (physostigmine; $50 \mu\text{M}$; $N = 6$) and pyridostigmine ($50 \mu\text{M}$; $N = 4$). In both intra- and extracellular recordings, synaptic responses were reversibly attenuated by these compounds. The intracellularly recorded EPSP was reduced from 18 ± 4 mV to $53 \pm 3\%$ of control during eserine (Fig. 3; washout: $94 \pm 8\%$; $N = 4$), indicating a similar degree of attenuation as attained by $100 \mu\text{M}$ carbachol. This effect was abolished by applying $1 \mu\text{M}$ atropine or $1 \mu\text{M}$ pirenzepine (Fig. 3). Thus, in the presence of an AChE inhibitor endogenous ACh induces a muscarinic receptor-mediated attenuation of the EPSP that is of similar strength as that obtained by exogenous cholinergic agonists.

3.4. Frequency-dependent modulation by muscarinic receptors

The strength of muscarinic modulation was examined across a range of stimulus rates. In the course of stimulus trains at rates above 1 Hz, the EPSP peak amplitude in the control series was observed to decline until a steady-state magnitude was reached after about eight stimuli (Fig. 4D). The values given here refer to this steady state. Fig. 4A shows that the EPSP peak amplitude was reduced with increasing frequencies in the control period, starting at 1 Hz and continuing until 12 Hz, which was the highest frequency tested. This phenomenon is termed 'frequency depression' [32].

During carbachol (10 μ M), EPSPs were clearly attenuated in the lower range of frequencies tested (0.25–4 Hz; Fig. 4B) but more weakly at the higher frequencies (8 and 12 Hz). At 8 and 12 Hz, the muscarinic modulation was still statistically significant at $P < 0.05$ with respect to the corresponding control values, but the difference was much more pronounced within the range of 0.25–4 Hz ($P < 0.005$; $N = 12$; Fig. 4C). Under carbachol conditions, frequency depression of the EPSP was much less conspicuous at rates up to 8 Hz, as expressed by the flattened shape of the fre-

Fig. 4. Frequency-dependent muscarinic modulation under current-clamp conditions. A: in the control situation, a gradual frequency-depression of the EPSP amplitude was found over the range of 0.25–12 Hz (indicated on the right). The traces shown are all taken after stabilization of the EPSP amplitude during the stimulus train. B: carbachol (10 μ M; right-hand column) strongly reduced EPSPs in the lower range of frequencies (0.25–2 Hz in this neuron), while there was no clear reduction at 4, 8 and 12 Hz. Calibration: 25 mV, 20 ms. C: frequency-response curve for the normalized and averaged EPSP peak amplitude under control (solid circles) and 10 μ M carbachol conditions (open circles); vertical bars represent standard errors of the mean. For each cell, the peak amplitude of the control EPSP at 0.25 Hz was set at 100% and synaptic responses evoked at different frequencies and under different drug conditions were normalized with respect to this value. D: afferent stimulation at frequencies > 1 Hz evoked a slow depolarization in addition to the fast EPSPs. The trace shown represents a slow depolarization with superimposed fast EPSPs (thin upward deflections; EPSPs were generally truncated due to a sample frequency of 200 Hz) in response to a stimulus train of 8 Hz under control conditions. The resting membrane potential was -80 mV. E: membrane potential changes under 8 Hz, carbachol conditions recorded in the same cell as shown in (D). The resting membrane potential remained at -80 mV. F: DC current (0.08 nA) was injected into the cell during carbachol application to compensate for the decrease in amplitude of the slow depolarization; a new stimulus train was applied under these conditions (upper trace; membrane potential was raised to -76 mV). Consequently, the absolute depolarizing level attained during the 8 Hz, carbachol train was now virtually equal to that during the 8 Hz, control train (lower trace, same as in D).

quency–response curve (Fig. 4C). No long-lasting changes were induced by repetitive stimulation, as indicated by the fact that recovery values closely approximated controls (not illustrated). Furthermore, the changes were found regardless of the precise order in which EPSPs evoked at different stimulus rates were monitored.

In the course of stimulus trains at a frequency above 1 Hz, the fast EPSPs appeared to be riding on a slow depolarization (Fig. 4D), the nature of which has not yet been investigated. Despite the limited amplitude of this slow response (up to 10 mV at 12 Hz), it cannot be assumed beforehand that it does not interfere with carbachol-induced changes in the frequency–response curve. Carbachol generally reduced the slow depolarization along with fast EPSPs (Fig. 4D,E). A trivial mechanism that might underlie the carbachol-induced flattening of the frequency–response curve would be a change in driving force for the EPSP as a consequence of the slow depolarization. During muscarinic receptor activation, this driving force may stay at a higher level because of the reduction of the slow depolarization. In theory, such a secondary effect might explain why EPSPs evoked at high stimulus rates are less attenuated by carbachol than those evoked at low rates.

This question was approached in two ways. Firstly, the decrease of the slow depolarization during carbachol was compensated for by injecting positive DC current into the cell during application of the stimulus train (Fig. 4D–F). The difference of muscarinic modulation at low and high frequencies remained as clear as under uncompensated conditions (five cells tested). Secondly, we investigated the frequency dependence of EPSCs under control and carbachol conditions using the whole-cell voltage-clamp technique (Fig. 5; $N = 6$). In the control period, EPSCs exhibited the same, low-pass type of frequency-dependence as encountered for EPSPs. During carbachol, EPSCs at low frequencies (0.25–2 Hz) were more strongly attenuated than at high frequencies (4–12 Hz), but there appeared to be a subtle difference with the curve obtained under current clamp conditions. The frequency curve under carbachol and voltage clamp conditions shows a slight peak in the relative EPSC amplitude at 4 Hz, which was significantly different from the 0.25 Hz, carbachol-value ($P < 0.05$; Fig. 5E). Under current clamp conditions, the relative EPSP amplitude at 4 Hz, carbachol was not significantly different from the 0.25 Hz, carbachol-value. Furthermore, the relative EPSC amplitudes at 0.25, 1 and 2 Hz significantly differed under control versus carbachol conditions ($P < 0.05$), whereas the values at 4, 8 and 12 Hz were not significantly different. Thus, the discriminating effect of carbachol on low versus high stimulus frequencies was more pronounced under whole-cell voltage clamp conditions as compared to current clamp conditions. In

Fig. 5. Frequency-dependent muscarinic modulation under voltage-clamp conditions. Control EPSCs evoked at 0.25 and 12 Hz are shown in (A) and (B), respectively (holding potential: -80 mV). Under carbachol conditions, the reduction of the EPSC was strong at 0.25 Hz (C) and much weaker at 12 Hz (D). Calibration: 200 pA, 25 ms. E: the normalized and averaged EPSC peak amplitude was plotted as a function of stimulus frequency. EPSCs under control and carbachol ($10 \mu\text{M}$) conditions are indicated by solid and open circles, respectively. S.E.M. values are represented by vertical bars. For method of normalization, see legend of Fig. 4.

conclusion, frequency-dependent muscarinic modulation appears to be unrelated to the effect of the slow depolarization seen in current clamp mode.

Another caveat might be that frequency depression is just less pronounced in synaptic responses of small amplitude, regardless of the degree of muscarinic receptor activation. However, despite the fact that our 0.25 Hz, control EPSPs exhibited a large variation in amplitude (range: 7–30 mV), a correlation between this amplitude and the extent of frequency depression was completely absent under control conditions. When the extent of frequency depression was expressed as the amplitude ratio of EPSPs at 12 Hz vs. 0.25 Hz, the orientation of the regression line was nearly horizontal and the correlation coefficient was 0.06 ($N = 12$; not shown). Consequently, it is warranted to reject this explanation.

In most reports on synaptic electrophysiology in the CNS, it has been stressed that excitatory synaptic responses exhibit facilitation over similar ranges of frequency as tested here [2,11,16,32]. Therefore, the frequency depression described above is considered an

interesting phenomenon by itself. In principle, the depression may be caused by endogenous modulators or by dynamic processes intrinsic to glutamatergic neurotransmission in the striatum. Repetitive stimulation of glutamatergic inputs to the ventral striatum may evoke release of endogenous ACh and could lead to muscarinic receptor-mediated modulation of EPSPs. Thus, the effects of exogenous muscarinic agonists may be occluded by those caused by endogenous ACh. However, application of atropine (1 μ M; six cells) did not alter the shape of the frequency–response curve. We next investigated to what extent frequency depression is caused by the release of other endogenous, EPSP-depressing modulators in the striatum, viz. GABA [26,27,30] and enkephalins [40]. The GABA_A-receptor coupled Cl[−] channel blocker picrotoxin (10 μ M; seven cells), the GABA_B receptor antagonist 2-hydroxy-saclofen (100 μ M; six cells) and the broad spectrum opiate receptor antagonist naloxone (1 μ M; $N=5$) all failed to induce a significant alleviation of frequency depression (not shown).

4. Discussion

Muscarinic receptor agonists exerted a clear inhibitory control over intracellularly recorded EPSPs in the ventral striatum and their attenuation was antagonized by atropine and pirenzepine. In extracellular recordings, carbachol depressed the population spike but not the compound action potential, indicating that fiber excitability remained intact. Furthermore, we have previously shown that the recorded EPSPs and population spikes are monosynaptic [26,27]. Thus, a reduced excitability of cortical neurons cannot account for our observations.

In a recent pharmacological study, part of which was conducted in the ventral striatum, Sugita et al. [34] ascribed muscarinic modulation of EPSPs to M3 receptors, whereas they also found an attenuation of IPSPs mediated by M1 receptors. A third aspect of muscarinic action in the ventral striatum may be a reduction of the inward-rectifying K⁺ conductance, which would be reflected by membrane depolarization in current-clamp recordings [35]. The lack of consistent postsynaptic depolarizations in our experiments may be due to the fact that we usually applied 10 μ M carbachol, whereas Uchimura and North [35] used a different agonist, viz. muscarine (1–100 μ M). Furthermore, the resting membrane potential of our cells was probably closer to the K⁺ reversal potential than in Uchimura and North's experiments. In agreement with our data, Dodt and Misgeld [13] and Akaike et al. [1] failed to detect significant changes in resting membrane potential or input resistance in the dorsal striatum with similar carbachol and muscarine concentrations as used here.

The muscarinic attenuation of EPSPs is most likely achieved by a mechanism located on the glutamatergic terminals, as previously suggested for hippocampal and dorsal striatal synapses (cf. [1,22,36]). The following findings provide arguments for a presynaptic modulation in the ventral striatum: (1) the attenuation of EPSPs is not accompanied by a change of resting membrane potential or a decrease in input resistance, excluding the possibility of a somatic or proximal dendritic shunt; (2) the time course of the EPSP is not affected by carbachol, whereas a distal dendritic shunt would be expected to result in a decrease of EPSP duration [15,31]; (3) carbachol leaves glutamate-induced depolarizations intact while suppressing EPSPs in the same cells (in agreement with findings by Sugita et al. [34]). Despite the fact that these findings are generally considered acceptable as arguments against a postsynaptic conductance change or an interaction with postsynaptic glutamate receptors, two cautionary notes should be added. Firstly, the strength of the argument based on EPSP time course depends on the amount of cable filtering in dendritic trees, but the extent to which this actually occurs in striatal neurons is unknown. Secondly, it cannot be excluded that the AMPA/kainate receptors mediating EPSPs are different from those generating glutamate-evoked depolarizations.

In response to application of the AChE inhibitors eserine and pyridostigmine, synaptic responses were attenuated in a manner indistinguishable from carbachol- or muscarine-induced modulation. The observed reduction of the EPSP was abolished by atropine and pirenzepine, indicating that endogenous ACh acted to reduce EPSPs by way of muscarinic receptors. Similar eserine-induced attenuating effects on EPSPs have been described in slices of the dorsal striatum [13]. In this structure, however, endogenous ACh may exert a tonically active control over synaptic transmission, since application of atropine alone markedly enhanced EPSPs, in contrast to our results in the ventral striatum [27].

4.1. Frequency-dependent modulation of synaptic transmission

The finding that the EPSP amplitude diminished with increasing stimulus rate under control conditions is interesting in the light of results obtained in many other mammalian brain areas, where EPSP facilitation instead of depression occurs over a similar range of frequencies [2,16,32]. In contrast, a frequency depression has been reported at 0.1 Hz and even lower rates for some invertebrate synapses, e.g., between sensory and motor neurons in *Aplysia* [6]. In the dorsal striatum, which is similarly organized as the ventral striatum, frequency depression has been reported for simi-

lar rates as used in the present study [7,13]. The mechanism underlying the depression may be intrinsic to the glutamatergic nerve terminal or may result from an action of an endogenously released neuromodulator. Here it was shown that the GABA_A receptor coupled Cl⁻ channel blocker picrotoxin, the GABA_B receptor antagonist 2-hydroxy-saclofen and atropine did not alter the frequency–response curve, indicating that endogenous GABA, ACh or enkephalins and related opioid receptor agonists cannot be held responsible for the depression. Even if the antagonist doses used here did not fully block their receptor/channel targets, a partial alleviation of frequency depression would have been expected, while our data showed no effect whatsoever. Other, as yet undefined endogenous modulators could mediate frequency depression, making it difficult at the present time to draw inferences about the underlying mechanism.

Under carbachol conditions, EPSPs were more strongly suppressed at the lower stimulus frequencies than at the high ones, resulting in a flattening of the frequency–response curve (Fig. 4). The frequency-dependence of this modulation appeared not to be due to an increased driving force during the stimulus train, as the differential reduction of EPSPs was maintained when compensatory current was injected into the cell during carbachol trials. Furthermore, the frequency-dependent effect was also observed under whole-cell voltage clamp conditions. In fact, the EPSC curve (Fig. 5) showed the frequency dependence more clearly than the current clamp recordings did. Another possible caveat, viz. that frequency depression would simply become weaker with smaller EPSP amplitudes, can be rejected due to the complete absence of correlation between the 0.25 Hz EPSP amplitude and the amount of frequency depression under control conditions. As yet it is unknown whether muscarinic modulation and frequency depression operate through the same mechanism, such that its activation by muscarinic receptors prevents further depression of the EPSP by repetitive stimulation.

In previously published literature on synaptic physiology of the CNS, we have found one indication for frequency-dependent modulation. In the rabbit nucleus accumbens studied *in vivo*, DeFrance et al. [12] noted an attenuating effect of dopamine on responses to stimulation of the fornix at a rate of 0.5 Hz, but not at 6.0 Hz. This finding suggests a similar tendency for frequency-dependent modulation as described here for the cholinergic system. However, the influence of additional dopamine effects and of slow depolarizations could not be examined in these field potential recordings. As concerns the present findings, however, we may conclude that frequency-dependent synaptic modulation by muscarinic receptors is non-trivial and can, as such, be added to the known repertoire of state

dependent actions of neuromodulators in integrated neural circuits. This point of general relevance can be further illustrated by considering propagation of activity through limbic cortex-ventral striatal circuitry. In the rat hippocampus, different modes of EEG activity have been reported in relation to behaviour [20,21,37], e.g., irregular slow activity in the awake, immobile rat and rhythmic slow activity (4–12 Hz) during 'voluntary' movements such as walking and rearing. Similar behaviour-related variations in EEG frequencies have been described for other parts of the limbic cortex [21]. As the ventral striatal projections of the subiculum and prefrontal cortex are thought to play an important role in planning and initiation of behavioural strategies [29], a frequency-selective processing of limbic inputs may differentially affect corticostriatal information flows associated with distinct overt acts (cf. [12]). Interestingly, the midline thalamic nuclei probably provide a strong excitatory drive for the cholinergic interneurons of the ventral striatum, as suggested by an ultrastructural study of Meredith and Wouterlood [23]. These nuclei, in turn, receive information from other limbic structures, such as the ventral subiculum and hypothalamus, and from the reticular formation [10,29].

In summary, acetylcholine is capable of exerting a powerful inhibitory control over excitatory limbic inputs to the ventral striatum by way of muscarinic receptors. The modulatory mechanism appears to be localized on glutamatergic terminals, raising the possibility of a selective attenuation of particular inputs while leaving postsynaptic excitability largely intact. The dependence of the modulation strength on stimulus frequency demonstrates a new type of activity dependence of neuromodulatory processes in the CNS and indicates that the effectiveness of muscarinic modulation of limbic inputs to the ventral striatum may be governed by the mode of rhythmic activity propagated through integrated thalamic-limbic circuits.

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