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ORIGINAL ARTICLE

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$GABA_B$ receptor-mediated modulation of the firing pattern of ventral tegmental area dopamine neurons in vivo

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Abstract Previous work demonstrates the fundamental role of the firing pattern, specifically the burst firing mode of midbrain dopamine (DA) neurons in the regulation of DA release. Spontaneous burst firing has been shown to be dependent upon NMDA receptor activation of the DA cells. In addition to NMDA receptors, previous studies have reported that also $GABA_B$ receptors modulate the firing pattern of DA neurons in the substantia nigra. In the present electrophysiological study the role of $GABA_B$ receptors in the modulation of the firing pattern of DA neurons in the ventral tegmental area (VTA) in anaesthetised Sprague-Dawley rats was analysed. Systemic administration of the selective and potent $GABA_B$ receptor agonist baclofen dose-dependently reduced firing rate and burst firing in VTA DA neurons. An increase in the regularity of DA cell firing was also observed. All these effects were effectively antagonized by administration of the selective $GABA_B$ antagonist CGP 35348 (100 mg/kg or 200 mg/kg, i.v.). Administration of CGP 35348 (400 mg/kg, i.v.) per se was associated with a long-lasting increase in burst firing activity. The effects of systemic administration of baclofen, alone or in combination with CGP 35348, on the firing rate were largely mimicked by local microiontophoretic application of the drugs onto the DA neurons.

Our findings indicate that central $GABA_B$ receptors may contribute to control of the burst firing mode of VTA DA neurons. Physiologically, activation of $GABA_B$ receptors may subserve a dampening function on VTA DA cell excitability which may counterbalance NMDA receptor-mediated excitation.

Keywords Baclofen · CGP 35348 · Mesolimbocortical · Microiontophoresis · Gamma aminobutyric acid

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Introduction

Ascending midbrain dopamine (DA) neurons are usually divided into two major cell groups, located in the substantia nigra zona compacta (SN) and the ventral tegmental area (VTA). These two nuclei comprise the origins of the nigrostriatal and mesocorticolimbic DA systems, respectively. The nigrostriatal DA system has been demonstrated to be critically involved in, e.g., motor control, whereas the mesocorticolimbic DA system plays a pivotal role in motivational and reward-related behavior and cognitive functions.

Electrophysiological studies in vivo have shown that both VTA DA and SN DA neurons are spontaneously active with two major modes of function: a slow irregular single spike firing mode and a relatively rapid burst firing mode with decreasing spike amplitude and increasing spike width within each burst (Grace and Bunney 1984a, 1984b). Previous studies emphasize that the firing pattern rather than the mean discharge rate of DA neurons is of critical importance for the control of DA release in terminal areas (see Gonon 1988; Bean and Roth 1991; Manley et al. 1992; Murase et al. 1993; Nissbrandt et al. 1994) as well as the effect of DA on postsynaptic target neurons, as shown by the expression of several immediate-early genes (Chergui et al. 1996, 1997). Burst firing appears to critically depend upon the afferent innervation of the DA neurons, since this functional mode is absent in DA neurons in the midbrain slice preparation, which is deprived of active inputs (Sanghera et al. 1984; Grace and Onn 1989; Seutin et al. 1990; Johnson et al. 1992). Furthermore, spontaneous burst firing in midbrain DA neurons has been shown to be driven by excitatory amino acid (EAA)-containing afferents, which originate in the prefrontal cortex

(Christie et al. 1985; Sesack et al. 1989), the subthalamic nucleus (Robledo and Féger 1990), the pedunculopontine nucleus (Di Loreto et al. 1992) and the habenula (Bayer and Pickel 1990) and act upon *N*-methyl-D-aspartate (NMDA) receptors on the DA neurons, both in the VTA and the SN (Charléty et al. 1991; Chergui et al. 1991; Johnson et al. 1992; Overton and Clark 1992; Zhang et al. 1992; Seutin et al. 1994; Wu et al. 1999).

In contrast to the relative wealth of information concerning the EAA-mediated excitation of DA neurons, comparatively less is known about inputs that may exert an inhibitory control of VTA DA neuronal firing patterns, e.g. inhibitory gamma amino butyric acid (GABA)-containing inputs. Quantitatively, GABAergic afferents represent the largest input to the midbrain DA neurons (Precht and Yoshida 1971; Bunney and Aghajanian 1976; Dray 1979; Gerfen 1985; Bolam and Smith 1990). These GABAergic neurons either form part of long loop systems, originating from rostral areas including the nucleus accumbens, striatum, globus pallidus, and ventral pallidum (Fonnum et al. 1978; Ribak et al. 1980; Walaas and Fonnum 1980), or represent intrinsic GABAergic interneurons in the VTA (Di Chiara et al. 1979; Stanford and Lacey 1996). Previous studies suggest that the $GABA_B$ receptor-mediated input to the VTA stems primarily from long loop mechanisms, whereas the $GABA_A$ receptor-mediated input emanates from intrinsic interneurons (Johnson and North 1992). GABA receptors have previously been shown to be involved in the regulation of the firing pattern of SN DA neurons (Johnson and North 1992; Engberg et al. 1993; Tepper et al. 1995; Erhardt et al. 1999). Thus, recent studies show that activation of somatodendritic $GABA_B$-receptors on SN DA neurons by microiontophoretic application of the $GABA_B$-receptor agonist baclofen causes cessation of burst firing and a pronounced regularization of the firing pattern of these neurons, albeit without any marked reduction of their average firing rate (Engberg et al. 1993; Erhardt et al. 1998). Previous studies of VTA DA neurons in the slice preparation demonstrate that activation of $GABA_B$ receptors exerts an inhibitory effect on the spontaneous pacemaker-like activity of these neurons (Johnson and North 1992; Seutin et al. 1994; Wu et al. 1999). In addition, other results in vivo show that systemic administration of the $GABA_B$ receptor agonist baclofen decreases the average firing rate of VTA DA neurons (Olpe et al. 1977). However, the putative modulation of VTA DA neuronal firing patterns by $GABA_B$ receptors in vivo remains to be investigated. Therefore, we have here studied the effects of two potent and selective $GABA_B$ receptor ligands, the $GABA_B$ receptor agonist baclofen, and the $GABA_B$ receptor antagonist CGP 35348 (Olpe et al. 1990), alone and in combination, on firing rate, burst firing and regularity of DA neurons in the VTA.

Materials and methods

Surgery and experimental procedures. Male albino rats (BKl:SD, i.e. Sprague-Dawley; Bantin and Kingman Universal, Sollentuna, Sweden) weighing 250–350 g were anesthetized with an initial dose of chloral hydrate (400 mg/kg, intraperitoneally) and maintained under surgical anesthesia throughout the experiments. A tracheal cannula was inserted to facilitate respiration. Rats were subsequently mounted in a stereotaxic frame (Kopf, Tujunga, Calif., USA). Rectal temperature was maintained at 37°C by means of a thermostatically controlled heating pad. A hole was drilled in the skull overlying the VTA, i.e. 0.7 mm lateral and 3.0 mm anterior to lambda (Paxinos and Watson 1998), and the dura mater was carefully removed. Drugs were delivered via a vein catheter inserted into either the jugular vein or a lateral tail vein. At the end of each experiment a negative current of 5 nA was passed through the electrode for 10 min to mark the recording site. Subsequently, rats were killed by overdose of anesthesia, brains were removed and stored in 25% sucrose in 10% formaldehyde. Finally, brains were sliced in 50-µm-thick sections and stained with neutral red. All recording sites included in this study were confirmed under microscope to be located within the VTA.

Extracellular recordings. Recording electrodes were pulled from glass capillaries in a vertical electrode puller (Narshige) and filled with 2 M sodium acetate saturated with Pontamine Sky Blue. The tips were broken back under microscope to an impedance of 3.0–6.0 MΩ measured at 135 Hz. Electrodes were lowered into the brain by means of a hydraulic microdrive (Kopf). A reference electrode was inserted into the subcutaneous tissue. Action potentials from single DA neurons in the VTA were visualized on an oscilloscope (Tektronix, TDS 310) allowing screen captures, and spikes were subsequently discriminated from background by means of a window discriminator. Discriminated spikes were fed to a computer and recorded on disk for subsequent analysis of the firing pattern (see below).

Microiontophoresis. In microiontophoretic experiments, the same experimental protocol was followed as above, with a few minor

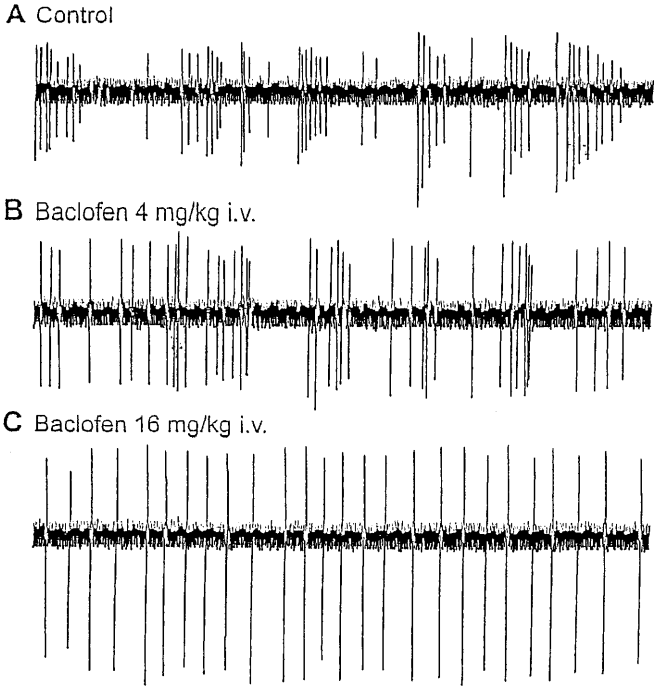

Fig. 1 Oscillographic traces of a typical VTA DA neuron **A** before and **B,C** after intravenous administration of baclofen. Relatively low doses of baclofen (4 mg/kg) selectively reduced burst firing and increased the regularity of firing (**B**), whereas high doses (16 mg/kg) also reduced the average firing rate and induced pacemaker-like firing (**C**)

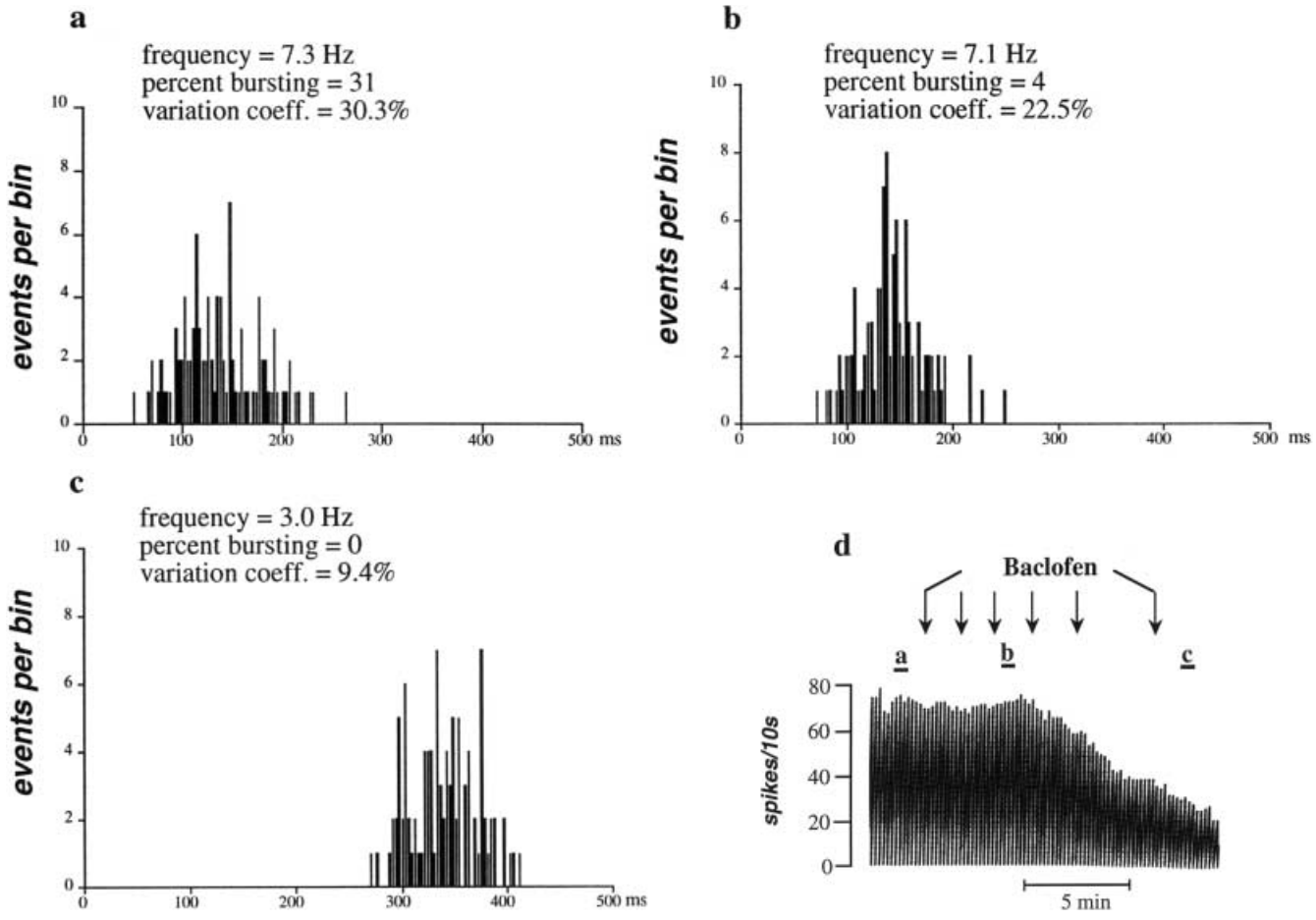

Fig. 2 Effects of baclofen on the firing pattern of a typical VTA DA neuron as shown by sequential interspike time-interval histograms (ISH:es; **a–c**). Baclofen dose-dependently reduced burst firing and increased the regularity of firing, as shown by a gathering of the ISH around the mean time interval. **d** Cumulative ratemeter histogram depicting the effect of baclofen (1+1+2+4+8+16 mg/kg, *at arrows*) on the firing rate. *Horizontal bars* indicate time periods where the three ISH:es were recorded

differences. Five-barrel microelectrodes, broken back to a tip diameter of ~3–8 μm were used. The central barrel was filled with 2 M NaCl saturated with Pontamine Sky Blue and was used for extracellular recording of action potentials. One of the four side barrels contained 4 M NaCl solution and was used for current balancing. The remaining barrels were filled with DL-baclofen (10 mM, dissolved in 0.15 M NaCl, pH 5.0), γ-aminobutyric acid (GABA; 50 mM, dissolved in 0.15 M NaCl, pH 3.5–4.0) and CGP 35348 (10 mM, dissolved in 0.15 mM NaCl, pH 3.75). The impedances of the five barrels were typically 2–7 MΩ in the central recording barrel and 40–90 MΩ in the side ejection barrels measured at 135 Hz in vitro. A retaining current of –5 nA was maintained between ejections. In all electrodes used, this current was sufficient to avoid leakage of drugs from the other side barrels since higher retaining currents did not affect the spontaneous firing rate of the neurons.

Identification of dopamine neurons and data analysis. Presumed DA neurons were found 7.5–8.5 mm from the brain surface and were recognized by their characteristic triphasic action potential waveforms of more than 2.0 ms duration, basal firing rates of 1–10 Hz and, in some neurons, the occurrence of burst firing (Grace and Bunney 1983).

The distribution of spikes was analysed on line utilising a Macintosh computer. The software used for the analysis of firing was written in-house using a high level object-oriented programming language called "G" (Lab VIEW; National Instruments, Austin, Tex., USA). The software was designed to sample and analyze the intervals of an arbitrary number of TTL pulses (corresponding to spikes passing through the discriminating filter) using a time resolution of 1 μs. An interspike interval was designated as the time (in ms) elapsed between the rising edge of two sequential TTL pulses. In order to avoid artifacts in the sampling procedure, the spike analyser ignored time intervals below 20 ms. The onset of a burst was determined as an inter-spike interval shorter than 80 ms and the termination of a burst by the next interval longer than 160 ms (Grace and Bunney 1984a, 1984b). Cells were considered to be bursting if at least one interspike time interval of 100 recorded spikes was below 80 ms. The software programme also sorted the intervals of 100 or 500 recorded spikes and divided them into 3-ms bins and displayed the results as an interspike time interval histogram (ISH) with regard to the number of intervals corresponding to each bin. The intervals were analysed with regard to the number of bursts that occurred during each 100-spike sampling period along with a calculation of the percentage of spikes fired in bursts. Firing rate, percentage of spikes fired in bursts, and variation coefficient (calculated as the ratio between the standard deviation and the mean interval of an ISH and used as a measure of the regularity of firing; Werner and Mountcastle 1963) were expressed as the median of at least three consecutive ISH:es. Only one DA neuron was studied in each animal.

Drugs. DL-baclofen and CGP 35348 (generous gifts from Novartis) were dissolved in 0.9% NaCl solution for intravenous (i.v.) injections.

Statistical analysis. Statistically significant differences regarding baclofen-induced effects on firing rate and variation coefficient were established using the Kruskal-Wallis analysis of variance followed by the Mann-Whitney *U*-test. For statistical analysis of burst firing activity, the Kruskal-Wallis analysis of variance followed by the Wilcoxon signed rank test were used. In addition, the effects of CGP 35348 per se on firing rate, burst activity and variation coefficient were statistically analyzed using the Kruskal-Wallis analysis of variance followed by the Wilcoxon signed rank test. Significance was assumed for all values where $P<0.05$.

Results

Systemic administration of the selective and potent $GABA_B$ receptor agonist baclofen (1–32 mg/kg, i.v.) was found to reduce the firing rate and the percentage of burst firing of VTA DA neurons (Figs. 1, 2, 4a,b). The average firing rate of VTA DA neurons was not significantly affected when baclofen was administered at doses ≤8 mg/kg. However, following higher doses, i.e. ≥16 mg/kg, a significant reduction of the average firing rate was observed (Figs. 1, 2, 4a). Administration of baclofen (4–32 mg/kg, i.v.) dose-

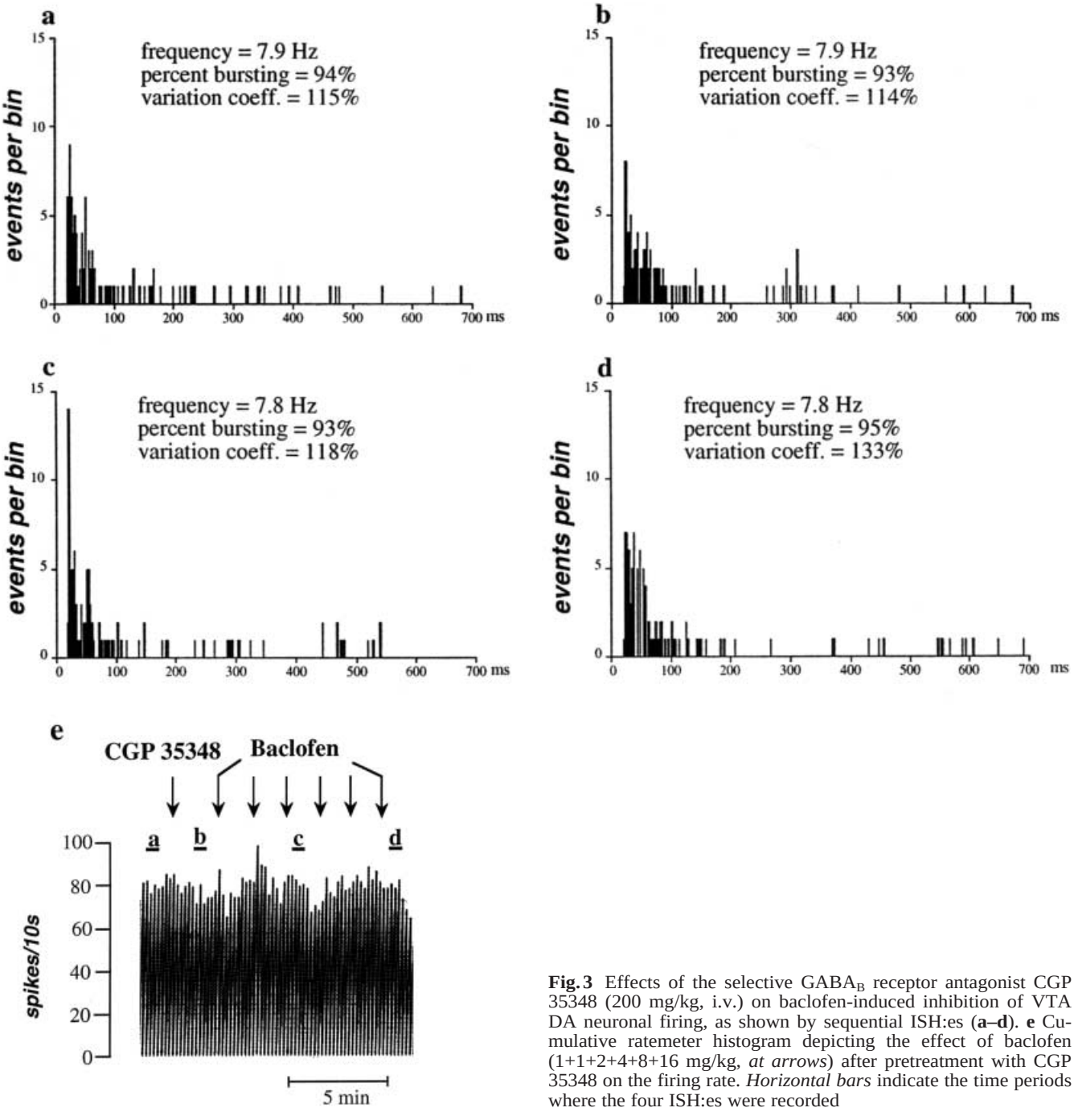

Fig. 3 Effects of the selective $GABA_B$ receptor antagonist CGP 35348 (200 mg/kg, i.v.) on baclofen-induced inhibition of VTA DA neuronal firing, as shown by sequential ISH:es (**a–d**). **e** Cumulative ratemeter histogram depicting the effect of baclofen (1+1+2+4+8+16 mg/kg, *at arrows*) after pretreatment with CGP 35348 on the firing rate. *Horizontal bars* indicate the time periods where the four ISH:es were recorded

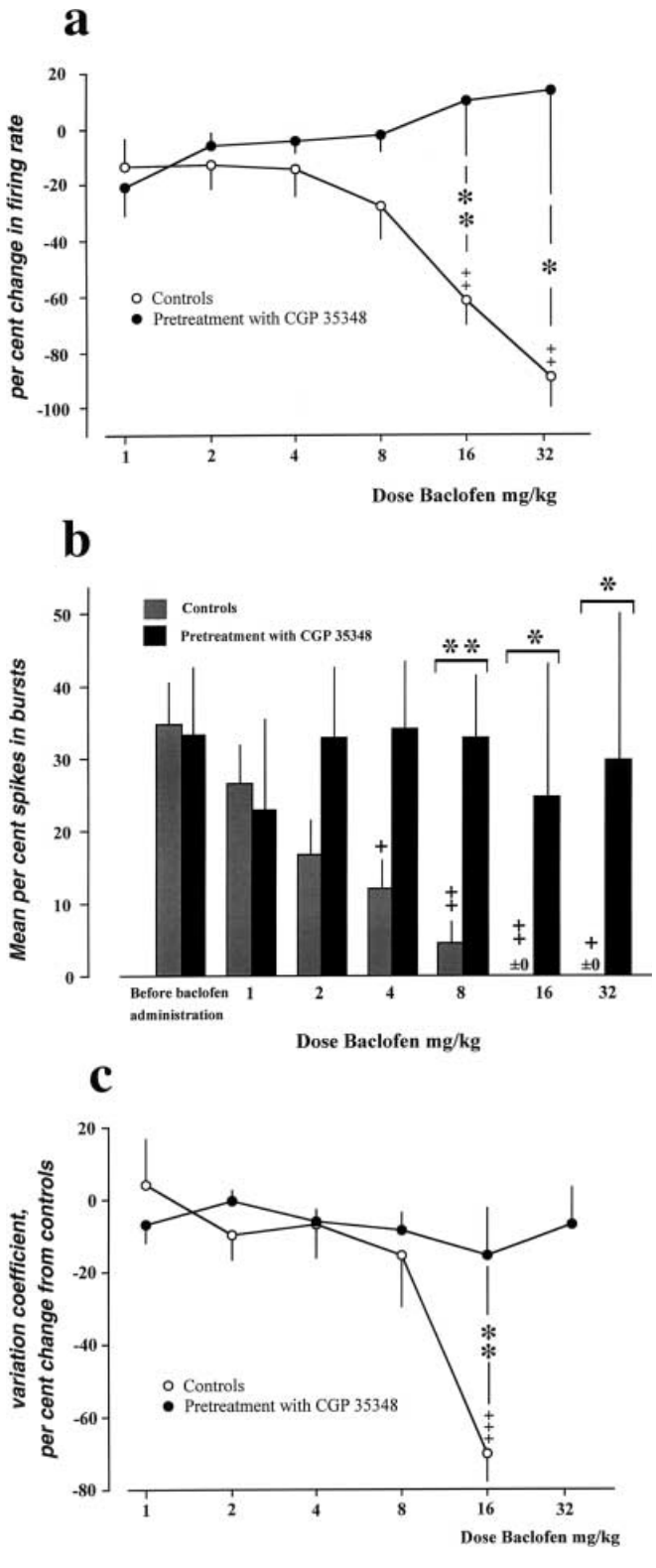

Fig. 4 **a** Cumulative dose-response curves illustrating the action of intravenously administered baclofen on the firing rate of VTA DA neurons in control rats and in rats pretreated with CGP 35348 (100 mg/kg or 200 mg/kg, i.v.). Each *point* respresents the mean ± SEM obtained from 4–13 neurons. **P*<0.05, ***P*<0.01, between-groups comparisons; ^{++}P<0.01 compared to predrug value (Mann-Whitney *U*-test). **b** Effect of intravenously administered baclofen on burst firing activity of VTA DA neurons in control rats and in rats pretreated with CGP 35348 (100 mg/kg or 200 mg/kg, i.v.). Each *bar* represents the mean ± SEM obtained from 4–13 neurons. **P*<0.05, ***P*<0.01, between-groups comparisons; ^{+}P<0.05, ^{++}P<0.01 compared to predrug value (Wilcoxon signed rank test). **c** Cumulative dose-response curves illustrating the action of intravenously administered baclofen on the regularity of firing, assessed by the variation coefficient of VTA DA neurons in control rats and in rats pretreated with CGP 35348 (100 mg/kg or 200 mg/kg, i.v.). Each *point* respresents the mean ± SEM obtained from 4–13 neurons. ***P*<0.01, between-groups comparison; ^{+++}P<0.001 compared to predrug value (Mann-Whitney *U*-test)

dependently reduced the percentage of burst firing (Figs. 1, 2, 4b). Thus, in 6 out of 12 neurons, burst firing was completely abolished following baclofen 8 mg/kg, and no burst firing was observed in any recorded DA neuron at doses ≥16 mg/kg i.v. Furthermore, the firing pattern of the VTA DA neurons was regularized following administration of baclofen at higher doses (≥16 mg/kg, i.v.), as indicated by a decreased variation coefficient (Figs. 1, 2, 4c). This regularization was extremely pronounced in some neurons, leading to a pacemaker-like firing pattern with a variation coefficient <10%, obtained at 32 mg/kg.

Pretreatment with the selective $GABA_B$ receptor antagonist CGP 35348 (100 mg/kg or 200 mg/kg, i.v., 5 min, *n*=13) antagonized all baclofen-induced effects, i.e. reduction in firing rate and burst firing as well as regularization of firing (Figs. 3, 4a–c).

In some but not all experiments, systemic administration of CGP 35348 (200–400 mg/kg, i.v.) alone induced a rather modest increase in firing rate of VTA DA neurons, an effect that was immediate in onset but rather short-lasting (<4 min; Table 1). On the other hand, intravenous administration of CGP 35348 (200–400 mg/kg) was always associated with a long-lasting increase in burst firing activity (Table 1). An increased burst firing activity, i.e. an increased number of bursts during a 100-spikes sampling period as well as an increased number of spikes within bursts, was observed in all spontaneously bursting DA neurons (*n*=7) following administration of CGP 35348 (400 mg/kg, i.v.). Furthermore, the only non-bursting neu-

Table 1 Effects of CGP 35348 on the firing rate and spike distribution of DA neurons in the VTA. Values are means ± SEM and obtained 1–2 min or 15 min after administration of CGP 35348

	Firing rate (Hz)	Variation coefficient	Spikes in bursts (%)
Predrug (*n*=11)	3.17±0.70	57.8±9.2	13.2± 8.2
CGP 35348 (200 mg/kg, 1–2 min, *n*=11)	3.55±0.62*	55.3±7.7	19.2± 8.0*
Predrug (*n*=8)	3.73±0.74	51.6±5.3	8.4± 4.9
CGP 35348 (400 mg/kg, 1–2 min, *n*=8)	3.74±0.73	58.4±3.0	18.3± 6.7*
CGP 35348 (400 mg/kg, 15 min, *n*=7)	4.12±0.88	74.1±4.9*	33.9±10.9*

**P*<0.05 (Wilcoxon signed rank test)

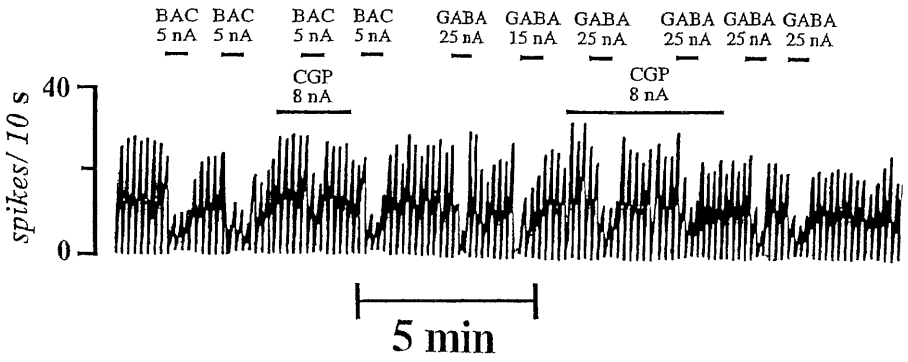

Fig. 5 Cumulative ratemeter histogram depicting the effects of microiontophoretic application of baclofen (*BAC*) or GABA on the firing rate of a typical VTA DA neuron and its antagonism by similarly applied CGP 35348 (*CGP*). *Horizontal bars* indicate ejection periods and the numbers above the ejection currents (nA)

ron found in this study was converted to a bursting neuron following this treatment. After 15 min, systemic administration of CGP 35348 also produced a dysregularized firing pattern, leading to an increased variation coefficient (Table 1).

Microiontophoretic application of baclofen or GABA produced a reduction of firing rate of VTA DA neurons (n=5; Fig. 5). Microiontophoretically applied CGP 35348 antagonized the reduction of firing rate caused by simultaneous microiontophoretic application of baclofen, whereas the antagonistic action of CGP 35348 on GABA-induced inhibition was less pronounced (Fig. 5). Microiontophoretic application of CGP 35348 per se did not significantly affect the firing pattern of VTA DA neurons.

Discussion

A major finding of the present study is that moderate activation of central $GABA_B$ receptors, as induced by the $GABA_B$ receptor agonist baclofen, may specifically suppress burst firing of VTA DA neurons. In addition, systemic injections of higher doses of baclofen also decreased the average firing rate, a finding in agreement with an earlier in vivo study (Olpe et al. 1977). Since the effects of systemic baclofen on firing rate and burst firing activity were effectively antagonized by the selective $GABA_B$ receptor antagonist CGP 35348, the baclofen-induced effects appear to be specifically related to activation of $GABA_B$ receptors.

A novel finding in this study is that high doses of the $GABA_B$ receptor antagonist CGP 35348 increased the burst firing activity and dysregularized the firing pattern of the VTA DA neurons. Thus, a tonically active $GABA_B$ receptor-mediated regulation of the burst firing of these neurons is, in principle, indicated. Such regulatory $GABA_B$ receptors are likely to be anatomically located somatodendritically on the VTA DA neurons. This view is supported by the finding that microiontophoretic administration of baclofen onto VTA DA neurons produces similar effects, i.e. decreased firing rate, as compared to systemic administration of the drug. Indeed, the inhibitory effect of $GABA_B$ receptor agonists on the firing rate of VTA DA neurons in vitro has previously been found to involve hyperpolarization of DA neuronal dendrites, as induced by an increased potassium conductance (see Seutin et al. 1994). Thus, the actions of systemically administered baclofen on VTA DA neurons could be occurring within the VTA itself. The lack of an increased burst firing of the VTA DA neurons following microiontophoretically applied CGP 35348 is probably related to the relatively low potency of the drug (Olpe et al. 1990) and/or in combination with a limited release of the drug from the microiontophoretic pipette (Ryall and Kelly 1978). Furthermore, in view of the general similarity between the functional characteristics of nigral and VTA DA neurons, and the fact that microiontophoretic application of another, highly selective and potent $GABA_B$ receptor antagonist, SCH 50911, indeed causes an activation of both firing rate and burst firing in SN DA neurons (Erhardt et al. 1998, 1999), an intra-VTA localization of the neuromodulatory effect of CGP 35348 seems likely.

The interactions between different afferent neuronal mechanisms modulating burst firing in VTA DA neurons remain to be completely understood. NMDA receptor stimulation has been shown to facilitate bursts of action potentials both in vivo (Charléty et al. 1991; Chergui et al. 1991; Zhang et al. 1992) and in vitro (Johnson et al. 1992; Overton and Clark 1992; Seutin et al. 1994; Wu et al. 1999). The present data, which demonstrate that $GABA_B$ receptor activation may even selectively suppress the burst firing mode in VTA DA neurons, suggest that this mechanism might serve to counterbalance the burst stimulation induced by NMDA receptor activation. Consequently, concomitant $GABA_B$ and NMDA receptor stimulation may not only contribute to the control of VTA DA neuronal activity in general, but also provide a machinery in vivo for an effective regulation of burst firing per se. This may be physiologically important, since DA neurons in the VTA are involved with transient and rapid changes in impulse activity, i.e. burst firing, in basic attentional and motivational processes which underlie learning and cognitive behavior (Schultz et al. 1993; Mirenowicz and Schultz 1996; Schultz 1998). Indeed, burst firing in midbrain DA neurons has previously been shown to be of specific importance for induction of expression of several immediate-early genes in DA target areas, e.g. the prefrontal cortex and nucleus accumbens (Chergui et al. 1996, 1997), an effect that seems to initiate long-term plasticity. Consequently, the activity of central $GABA_B$ receptors

may contribute to modulate DA-regulated behavioral functions.

Previous studies have provided considerable evidence to indicate that mesolimbic DA neurons are critically involved in reward mechanisms, including nicotine reinforcement (see Koob 1992). Moreover, systemic nicotine has been shown to preferentially stimulate the burst firing mode of midbrain DA neurons (Grenhoff et al. 1986; Erhardt et al. 2001). Therefore it is significant that baclofen has recently been found to attenuate the reinforcing properties of nicotine in self-administering rats (Corrigall et al. 2000; G. Mereu, personal communication). Under these conditions, baclofen might thus reduce the reinforcing properties of nicotine by suppressing the nicotine-induced stimulation of burst firing in VTA DA neurons.

Our previous studies showed that impairment of the glutamatergic input to the mesocorticolimbic DA neurons in the VTA is associated with profoundly distorted firing patterns of these cells, a dysfunctional state of the DA system that may have bearing on the pathophysiology of schizophrenia (Svensson and Tung 1989; Svensson et al. 1995; see Svensson 2000). The results of the present study suggest that impairment of the GABAergic input to the VTA may also contribute to distorting signaling in the mesocorticolimbic dopamine system in association with psychotic disorders.

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