Presynaptic $GABA_B$ and adenosine A_1 receptors regulate synaptic transmission to rat substantia nigra reticulata neurones

Ke-Zhong Shen* and Steven W. Johnson*†‡

*Department of *Physiology and Pharmacology and †Department of Neurology, Oregon Health Sciences University, Portland, OR 97201, USA*

1. Patch pipettes were used to record whole-cell currents under voltage clamp in substantia nigra zona reticulata (SNR) neurones in the rat midbrain slice. Bipolar electrodes evoked synaptic currents mediated by glutamate (EPSCs) and $GABA_A$ receptors (IPSCs).

2. Baclofen reduced the amplitude of IPSCs by 48% at its IC_{50} value of 0·60 μM. The $GABA_B$ antagonist CGP 35348 blocked this effect with a K_d value estimated by Schild analysis of 5 μM.

3. Adenosine reduced IPSCs by 48% at its IC_{50} value of 56 μM. Adenosine agonists reduced IPSCs with the following rank order of potency: CPA (N^6-cyclopentyladenosine) > R-PIA ($R(-)N^6$-(2-phenylisopropyl)adenosine) > CHA (N^6-cyclohexyladenosine) = NECA (5′-*N*-ethylcarboxamidoadenosine) > 2-CADO (2-chloroadenosine) > adenosine. Schild analysis yielded a K_d value of 0·4 nM for antagonism of CPA by the adenosine A_1 receptor antagonist DPCPX (8-cyclopentyl-1,3-dipropylxanthine).

4. Both baclofen and adenosine reduced the magnitude of paired-pulse depression of IPSCs, and neither blocked currents evoked by GABA, which was pressure-ejected from micropipettes.

5. Glutamate EPSCs were reduced by baclofen (IC_{50} = 0·78 μM) and adenosine (IC_{50} = 57 μM). Schild analysis yielded a K_d value of 11 μM for antagonism of baclofen-induced inhibition of EPSCs by CGP 35348. DPCPX (1 μM) completely blocked the inhibitory effects of adenosine (100 μM) and CPA (100 nM) on EPSCs. Neither adenosine nor baclofen reduced inward currents evoked by glutamate which was pressure-ejected from micropipettes.

6. These results show that presynaptic $GABA_B$ and A_1 receptors reduce glutamate and GABA release from nerve terminals in the SNR.

The substantia nigra zona reticulata (SNR) is a midbrain nucleus comprising GABA-containing neurones that are located lateral to the dopamine-containing cells of the substantia nigra zona compacta (SNC) (Parent, Côté & Lavoie, 1995). The SNR receives a massive GABA-containing pathway from striatum, a smaller input containing GABA from globus pallidus, and a glutamate- containing pathway from subthalamic nucleus (Smith & Bolam, 1990). The SNR projects axons to the ventral thalamus and superior colliculus, and thereby serves as a major output nucleus for the basal ganglia (Yasui, Tsumori, Ando & Domoto, 1995). By connecting the striatum to the thalamus, the SNR is an important relay network that can be modulated by excitatory input from subthalamic nucleus and inhibitory input from globus pallidus. It is well established that overactivity of SNR neurones contributes to symptoms of Parkinson's disease (DeLong, 1990) and also facilitates the spread of seizure discharges in models of epilepsy (Gale, 1985). Thus abnormal regulation of SNR neurones contributes significantly to some common neurological problems.

Binding sites for adenosine A_1 and $GABA_B$ receptors are relatively dense in the SNR (Bowery, Hudson & Price, 1987; Fastbom, Pazos & Palacios, 1987). Because these receptor types modulate synaptic transmission in many central nuclei (Chesselet, 1984), the present study was undertaken to test the hypothesis that adenosine and baclofen regulate synaptic inputs to SNR neurones. Using patch pipettes to record whole-cell currents in the midbrain slice, the goals of

‡ To whom correspondence should be addressed at the Department of Physiology and Pharmacology, L-334, Oregon Health Sciences University, 3181 S. W. Sam Jackson Park Road, Portland, OR 97201, USA.

this study were (1) to identify the receptor pharmacology of synaptic currents recorded in SNR neurones, and (2) to characterize receptor-mediated effects of adenosine and baclofen on excitatory and inhibitory synaptic transmission. Some of these results have been presented in abstract form (Shen & Johnson, 1996).

METHODS

Tissue preparation

Sprague–Dawley rats (8–24 days old; Bantin & Kingman, WA, USA) were anaesthetized with halothane and killed by severing major thoracic vessels. The brain was rapidly removed and horizontal slices (300 μm) containing the midbrain were prepared as described previously (Wu, Mercuri & Johnson, 1995). Briefly, slices were cut in a vibratome in cold physiological saline and placed on a supporting net in a recording chamber (volume 500 μl). The slices were immersed and perfused with a flowing (2 ml min^{-1}) saline solution that contained (mM): 126 NaCl, 2·5 KCl, 2·4 $CaCl_2$, 1·2 $MgCl_2$, 1·2 NaH_2PO_4, 19 $NaHCO_3$ and 11 glucose. This solution was saturated with 95% O_2 and 5% CO_2, and had a pH of 7·35 at 35–37 °C. Using a dissection microscope for visual guidance, recordings were made from the SNR 1–2 mm lateral to the substantia nigra zona compacta.

Electrophysiological recordings

Whole-cell tight-seal recordings were made with pipettes containing (mM): 130 potassium gluconate, 1 NaCl, 2 $MgCl_2$, 1 $CaCl_2$, 11 EGTA, 10 Hepes, 1·5 ATP and 0·3 GTP. The pH of the internal solution was adjusted to 7·3–7·4 with KOH. Membrane currents were recorded under voltage clamp (holding potential −60 mV) and amplified with an Axopatch-1B amplifier. Data were acquired and analysed using pCLAMP software, a Digidata analog/digital interface (Axon Instruments), and an IBM-compatible personal computer. Holding currents were recorded continuously using a MacLab analog/digital interface, Chart software (AD Instruments, Castle Hill, Australia), and a Macintosh IIVX computer. Series resistance was electronically compensated 50–80% to 10–30 MΩ; membrane potentials have been corrected for the liquid junction potential (10 mV).

Synaptic currents

Bipolar stimulation electrodes (tip separation 300–500 μm) were made from electrolytically sharpened tungsten wire and their tips were placed in the slice within 500 μm of the recording electrode. A single rectangular pulse (0·1 ms duration) of constant current was used to evoke a synaptic current every 30 s. An inhibitory postsynaptic current mediated by $GABA_A$ receptors was isolated pharmacologically by recording in the presence of (±)-2-amino-5-phosphonopentanoic acid (AP5; 50 μM) and 6-cyano-7-nitroquinoxalone (CNQX; 10 μM), which block NMDA and non-NMDA receptors, respectively. Studies on excitatory postsynaptic currents were done in the presence of bicuculline (30 μM) to block $GABA_A$ IPSCs. To control for changes in amplitude of synaptic currents that can occur gradually over time, currents recorded during drug application were always compared with the average of currents recorded before and after recovery from the drug. The synaptic currents shown in the figures represent the average of three responses.

Drugs

Stock solutions of drugs were diluted to the desired concentration in perfusate immediately prior to their use. A stock solution of dopamine was kept on ice to retard oxidation. Approximately 30 s was required for the drug solution to enter the recording chamber; this delay was due to passage of the perfusate through a heat exchanger. In some experiments, GABA (10 mM) and glutamate (10 mM) were ejected by pressure from glass micropipettes using a Picospritzer II pressure-ejection unit (General Valve Corp., Fairfield, NJ, USA). Pressure-ejection pulses lasted 100–700 ms, depending on the depth of neurone under study. Tips of pressure-ejection pipettes (1 μm in diameter) were placed approximately 100 μm above the surface of the brain slice. Constant pressure (35–140 kPa) was used to eject drug solutions from pipette tips. Adenosine, baclofen hydrochloride, bicuculline methiodide, GABA, dopamine hydrochloride, glutamate monosodium and [Met^5]-enkephalin were obtained from Sigma Chemical Co. AP5, 2-CADO (2-chloroadenosine), CGS 21680 (2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxy-amidoadenosine), CHA (N^6-cyclohexyladenosine), CNQX, CPA (N^6-cyclopentyladenosine), DPCPX (8-cyclopentyl-1,3-dipropylxanthine), NECA (5′-*N*-ethylcarboxamidoadenosine) and R-PIA ($R(-)N^6$-(2-phenylisopropyl)-adenosine) were obtained from Research Biochemicals International (Natick, MA, USA). CGP 35348 was a gift from Ciba-Giegy (Basel, Switzerland).

Data analysis

Using the KaleidaGraph curve-fitting program (Synergy Software, Reading, PA, USA) on a Macintosh computer, concentration–response curves were fitted to the equation: $y = ax/(x + b)$, where y is the drug effect, a is maximum effect, x is concentration of drug and b is the IC_{50} value (concentration of drug producing 50% of maximum inhibition). An IC_{50} value for a drug was calculated for each neurone, and the mean IC_{50} (and S.E.M.) was obtained by averaging results from several neurones. Two-way repeated measures analysis of variance (ANOVA) was used to test differences in concentration–response curves for statistical significance (SigmaStat, Jandel Scientific, San Rafael, CA, USA). Two-tailed Student's t tests were used to evaluate whether or not differences between IC_{50} values were statistically significant. Numerical data presented in the text are expressed as means ± S.E.M.

Schild analysis

The method of Schild was used to calculate the dissociation equilibrium constant (K_d) for receptor antagonists (Schild, 1957). Although concentrations of antagonist drugs were increased cumulatively, the effect of each concentration of agonist was allowed to wash out completely before the next concentration was applied. Antagonism was assumed to be competitive, and each estimate of K_d was obtained by fitting the data to a straight line with a slope of 1, and extrapolating the line to the X-intercept.

RESULTS

Membrane properties of SNR neurones

Electrophysiological studies on membrane properties suggest that the SNR is composed of two types of neurone. Neurones were categorized according to the magnitude of hyperpolarization-activated time-dependent inward current (I_h). The most common type of neurone (73 of 86 cells; 85%) exhibited little or no I_h (58 ± 4 pA) in response to a 400 ms hyperpolarizing voltage step (from −70 to −130 mV), as seen in Fig. 1*A*. Instead, these cells showed significant time-independent inward rectification at hyperpolarizing potentials; for example, whole-cell chord conductance was 5·9 ± 0·4 nS ($n = 25$) when measured at relatively hyperpolarized potentials (−120 to −130 mV), whereas

conductance decreased to 1·9 ± 0·2 nS when measured at less hyperpolarized potentials (−70 to −80 mV) in the same neurones. Most of these neurones (46 of 73 cells; 63%) fired spontaneous action potentials at zero holding current; the remainder had an average resting potential of −67 ± 1 mV ($n = 27$). Action potentials were relatively narrow with a duration < 1·5 ms. Baclofen (10 μM), a $GABA_B$ agonist, produced a small outward current in some neurones (15 ± 3 pA at −70 mV; $n = 18$), but no current was evoked in more than half of those tested (26 of 44 cells) (Fig. 1*B*). Perfusion with dopamine (30 μM; $n = 4$), quinpirole (10 μM; $n = 3$) and [Met^5]enkephalin (10 μM; $n = 4$) had no effect on the holding current of these neurones, which agrees with the results of others (Rick & Lacey, 1994). Results of previous studies have shown that the physiological and pharmacological profiles of these cells are consistent with the principal GABA-containing output neurone of the SNR (Nakanishi, Kita & Kitai, 1987; Hajós & Greenfield, 1994; Stanford & Lacey, 1996).

The second neuronal type in the SNR was less common (13 of 86 neurones); they were characterized by relatively large I_h (470 ± 40 pA; $n = 13$) evoked by a 60 mV hyperpolarizing voltage step (from −60 to −120 mV; Fig. 1*C*), and all cells spontaneously fired broad (> 2 ms) action potentials at 1–5 Hz when no holding current was applied ($n = 13$). Baclofen (10 μM) evoked a large outward current at −60 mV (270 ± 45 pA; $n = 4$), as did dopamine (30 μM) and the dopamine D_2 agonist quinpirole (Fig. 1*D*). These electrophysiological and pharmacological characteristics are identical to those of dopamine-containing neurones in the adjacent SNC (Lacey, Mercuri & North, 1988; Yung, Häusser & Jack, 1991), and dopamine-containing neurones are known to be scattered throughout the SNR (Palkovits & Jacobowitz, 1974). Therefore, it is likely that this subpopulation of neurones in the SNR are dopamine containing.

In order to focus on the main output neurone of the SNR, results from the presumed dopamine-containing neurone were excluded. Thus all results presented in the remainder of this study were obtained from the principal neuronal type in the SNR.

Studies on $GABA_A$ IPSCs

Baclofen inhibits IPSCs. In the presence of AP5 (50 μM) and CNQX (10 μM), a single stimulus delivered to the slice evoked an IPSC with an amplitude of 245 ± 25 pA (range 42–426 pA, $n = 32$). This IPSC was mediated by $GABA_A$ receptors because it was completely blocked by bicuculline (30 μM; Fig. 2*A*). Baclofen reduced the amplitude of $GABA_A$ IPSCs in a concentration-dependent manner with an IC_{50} of 0·60 ± 0·08 μM ($n = 19$; Fig. 2*B*). The minimal effective concentration was 30 nM, and 10 μM baclofen reduced IPSCs by 84 ± 3% ($n = 7$). Baclofen reached its peak effect within 2 min. Effects of baclofen were completely reversible 5–10 min after washout.

CGP 35348 (300 μM), a $GABA_B$ receptor antagonist, reversibly blocked the baclofen-induced inhibition of $GABA_A$ IPSCs. Increasing concentrations of the antagonist (10–300 μM) progressively shifted the baclofen concentration–response curve to the right (Fig. 2*B*). The baclofen IC_{50} was 2·0 ± 0·5 μM in 10 μM CGP 35348 ($n = 3$), 5·1 ± 1·4 μM in 30 μM CGP 35348 ($n = 4$),

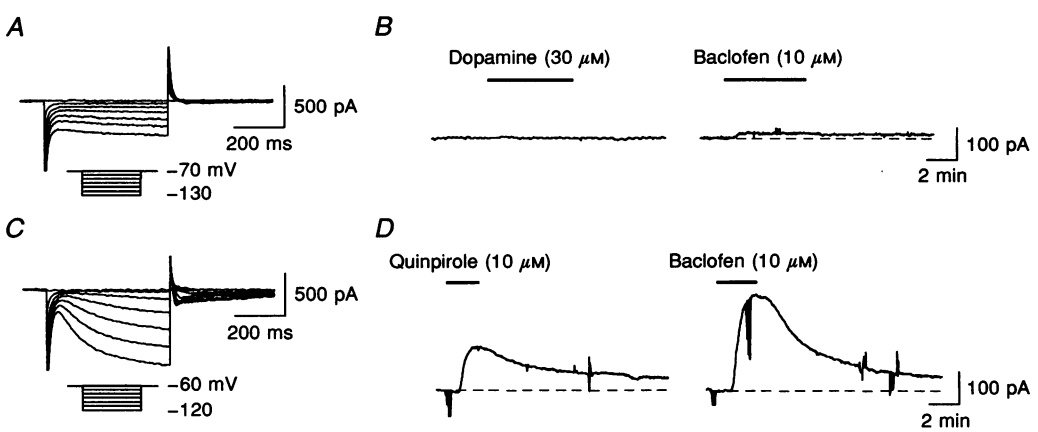

Figure 1. Two types of neurone in the SNR

A, membrane currents recorded during a series of hyperpolarizing voltage steps (inset) in the most common type of SNR neurone. This cell type exhibits inward rectification, but time-dependent inward current (I_h) was relatively small. *B*, currents recorded at −70 mV in the same type of neurone shown in *A*. Dopamine evokes no outward current, while baclofen evokes about 15 pA. *C*, membrane currents recorded during a series of hyperpolarizing voltage steps in the less common type of SNR neurone. This cell type exhibits a prominent I_h. *D*, current traces recorded from the same neuronal type as in *C*; both quinpirole and baclofen evoke significant outward currents (at −60 mV). Data presented in *A* and *B* are characteristic of the principal GABA-containing output neurone of the SNR, whereas data presented in *C* and *D* are characteristic of dopamine-containing neurones. The interrupted lines in *B* and *D* indicate zero holding current.

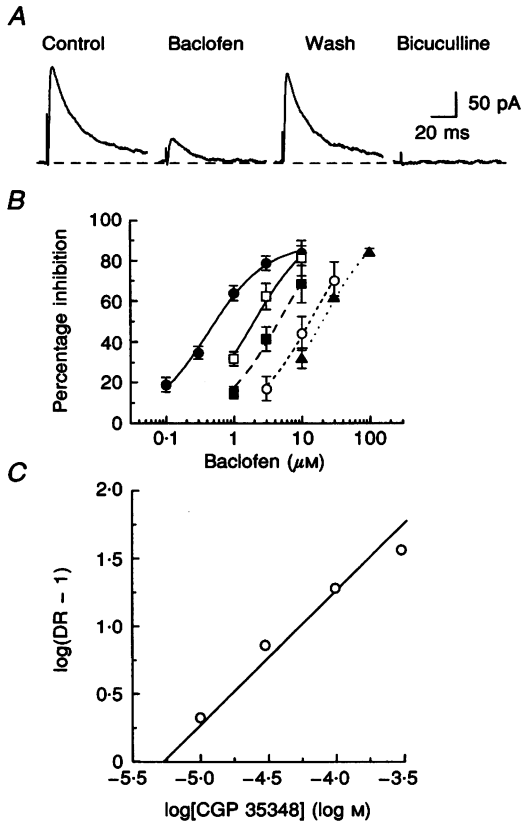

Figure 2. Baclofen inhibits $GABA_A$ IPSCs in SNR neurones

A, baclofen (1 μM) reversibly reduces the amplitude of a $GABA_A$ IPSC. This synaptic current, which was recorded in the presence of AP5 (50 μM) and CNQX (10 μM), was completely blocked by the $GABA_A$ receptor antagonist bicuculline (30 μM). The interrupted line indicates zero holding current. *B*, baclofen concentration–response curves for inhibition of IPSCs in the absence and presence of four different concentrations of CGP 35348 (●, control; □, 10 μM; ■, 30 μM; ○, 100 μM; ▲, 300 μM). The control IC_{50} for baclofen is 0·60 ± 0·08 μM ($n = 19$). In the presence of CGP 35348, each data point represents the mean (± S.E.M.) of 3–5 neurones. *C*, Schild plot of data shown in *B* yields a K_d of 5 μM for CGP 35348. The dose ratio (DR) is defined as the IC_{50} of baclofen in the presence of antagonist divided by its control IC_{50}.

12·5 ± 2·4 μM in 100 μM CGP 35348 ($n = 4$), and 21·8 ± 5·6 μM in 300 μM CGP 35348 ($n = 3$). Using results from the Schild plot analysis shown in Fig. 2*C*, the K_d (dissociation constant) for CGP 35348 was 5 μM.

In order to test the site of action of baclofen, its effects were investigated on outward currents evoked by GABA, which was ejected by pressure from micropipettes. Focal application of GABA evoked transient outward currents (50–150 pA) that were more than 90% blocked by bicuculline (30 μM) (Fig. 3*A*). Baclofen (1 μM) failed to inhibit currents evoked by GABA ($n = 5$), suggesting that baclofen does not inhibit IPSCs by a postsynaptic mechanism. To explore further the site of action of baclofen, pairs of

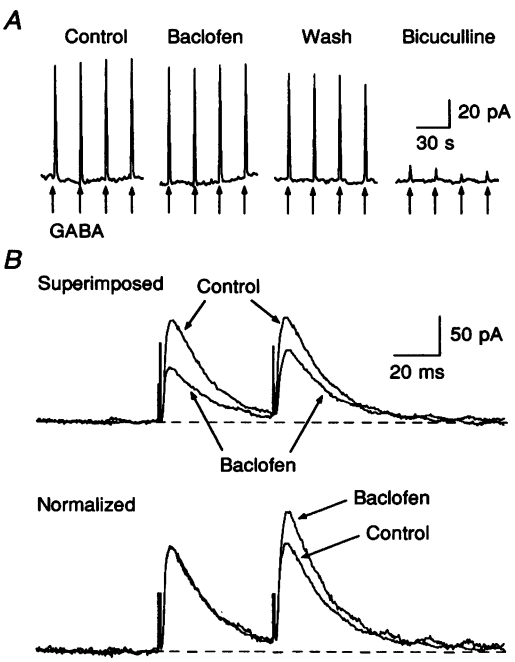

Figure 3. Baclofen acts presynaptically to inhibit $GABA_A$ IPSCs

A, currents evoked by exogenous GABA are almost completely blocked by bicuculline (30 μM) but not by baclofen (1 μM). The upward arrows indicate the times at which GABA was ejected by pressure from the tip of a micropipette placed near the recording pipette. *B*, upper traces show superimposed IPSCs evoked by pairs of stimuli (50 ms apart) in the absence (control) and presence of baclofen (100 nM). Note that the first IPSC is smaller in the presence of baclofen, but the second IPSC is larger than the first. The lower traces are the same as those above, except that the amplitude of the first IPSC recorded in baclofen has been normalized to the first IPSC recorded under control conditions.

stimuli delivered 50 ms apart were used to evoke IPSCs (Fig. 3*B*). In the absence of baclofen, the ratio of the amplitude of the second IPSC divided by the first was 0·95 ± 0·05 ($n = 5$). However, baclofen (300 nM) increased the IPSC paired-pulse ratio to 1·32 ± 0·15 in the same neurones ($P < 0·01$, paired t test). Furthermore, baclofen increased the amplitude of the second $GABA_A$ IPSC even though the amplitude of the first was reduced compared with control amplitude (Fig. 3*B*). These data are consistent with the conclusion that baclofen acts presynaptically to inhibit GABA release from nerve terminals (Davies, Davies & Collingridge, 1990).

Adenosine inhibits IPSCs. Adenosine reduced the amplitude of $GABA_A$ IPSCs in a concentration-dependent manner, as seen in Fig. 4*A*. Moreover, the adenosine A_1 receptor antagonist DPCPX (100 nM) shifted the adenosine concentration–response curve to the right (Fig. 4*A* and *B*). In the absence of DPCPX, the IC_{50} for adenosine-induced inhibition of IPSCs was 56 ± 10 μM ($n = 11$), whereas it was 650 ± 100 μM ($n = 4$) in 100 nM DPCPX. As seen in Fig. 4*B*, adenosine produced a threshold effect at 3 μM, whereas 1 mM reduced the IPSC amplitude by 86 ± 7% ($n = 4$). Effects of adenosine began within 1 min of perfusion, reached a steady state in 5 min, and washed out completely 5–10 min later. Despite the prominent effect on IPSCs, adenosine (up to 1 mM) had no effect on holding current in SNR neurones.

Adenosine A_1 receptor agonists also reduced $GABA_A$ IPSCs in a concentration-dependent fashion (Fig. 5*A*). CPA was the most potent, with an IC_{50} of 0·060 ± 0·014 μM ($n = 17$). R-PIA was also relatively potent ($IC_{50} = 0·20 ± 0·13$ μM, $n = 4$), but its maximum effect (42 ± 12% reduction in IPSC amplitude) was significantly less than those of adenosine and NECA ($P < 0·05$, one-way ANOVA on ranks). The IC_{50} of CHA (0·31 ± 0·10 μM, $n = 4$) was similar to that of NECA (0·48 ± 0·09 μM, $n = 3$); 2-CADO was the least potent of these agonists ($IC_{50} = 4·0 ± 0·6$ μM, $n = 3$). Beginning with the most potent, the rank order for potency of adenosine agonists was: CPA > R-PIA > CHA = NECA > CADO > adenosine.

Compared with adenosine, the higher potency of CPA permitted a more complete study of the antagonistic effects of DPCPX. As seen in Fig. 5*B*, increasing concentrations of DPCPX progressively shifted the CPA concentration–response curve to the right. DPCPX at 10 nM increased the IC_{50} of CPA to 1·4 ± 0·6 μM ($n = 5$), 100 nM increased the IC_{50} to 9·3 ± 2·0 μM ($n = 5$), and 300 nM DPCPX increased the IC_{50} to 60 ± 13 μM ($n = 3$). Schild plot analysis of these data (Fig. 5*C*) yielded a K_d value of 0·4 nM for DPCPX.

When DPCPX alone was added to the perfusate (100 nM), this caused the amplitude of $GABA_A$ IPSCs to increase by 13 ± 2% ($n = 7$; $P < 0·001$, paired t test). This suggests that endogenous adenosine exerts a tonic inhibitory influence on GABA release in the brain slice. In contrast, the selective adenosine A_2 receptor agonist CGS 21680 (10 μM) had no effect on the amplitude of $GABA_A$ IPSCs ($n = 3$). Membrane holding current was not altered by DPCPX or CGS 21680.

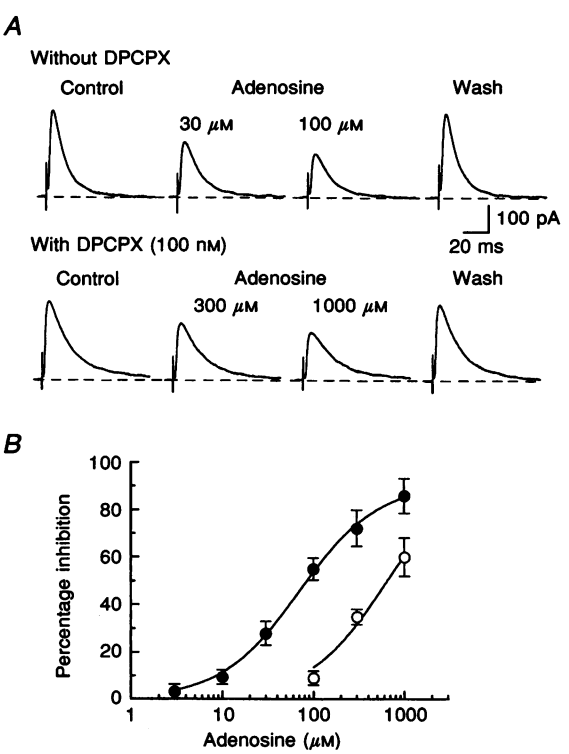

Figure 4. Antagonism of adenosine-induced inhibition of $GABA_A$ IPSCs by DPCPX

A, DPCPX (100 nM) reduces the potency of adenosine for inhibiting IPSCs. In the absence of DPCPX, 30–100 μM adenosine is required to reduce the IPSC by 50%. However, in the presence of DPCPX, 300–1000 μM adenosine is required to reduce IPSCs to a similar extent. *B*, the adenosine concentration–response curve for inhibition of IPSCs is shifted to the right in DPCPX (100 nM). In the absence of DPCPX (●), the adenosine IC_{50} is 56 ± 10 μM ($n = 11$). In the presence of DPCPX (○), the adenosine IC_{50} is 650 ± 100 μM ($n = 4$).

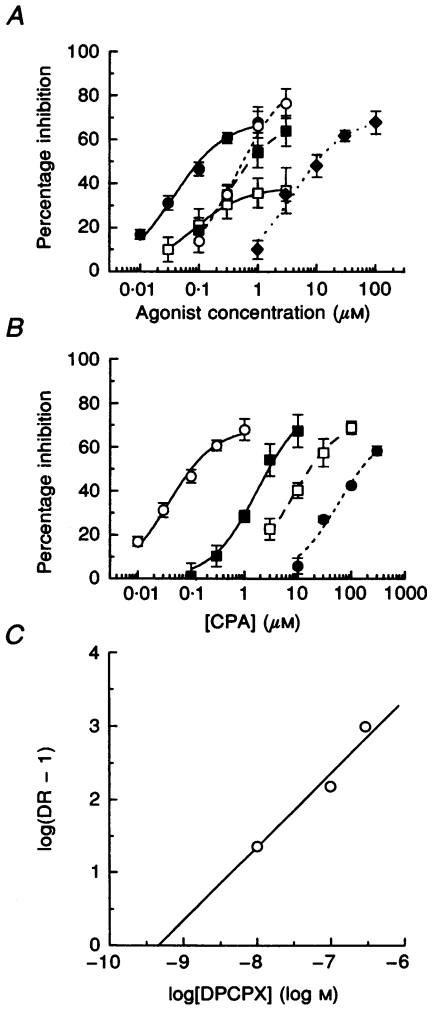

Figure 5. Adenosine agonists mimic the actions of adenosine to inhibit $GABA_A$ IPSCs

A, concentration–response curves for inhibition of IPSCs by adenosine agonists (●, zero; □, R-PIA; ■, CHA; ○, NECA; ◆, 2-CADO). CPA was the most potent (IC_{50} = 0·060 ± 0·014 μM, n = 17), whereas 2-CADO was the least potent (IC_{50} = 4·04 ± 0·576 μM, n = 3). The percentage maximum inhibition by R-PIA was significantly less than that of of NECA ($P < 0·05$; one-way ANOVA on ranks). Each data point is the mean ± S.E.M. of 3–17 neurones. *B*, increasing concentrations of DPCPX (○, zero; ■, 10 nM; □, 100 nM; ●, 300 nM) progressively shift the CPA concentration–response curve to the right. Each data point is the mean ± S.E.M. of 3–17 neurones. *C*, Schild plot of data shown in *B* gives an estimate of 0·4 nM for the K_d of DPCPX.

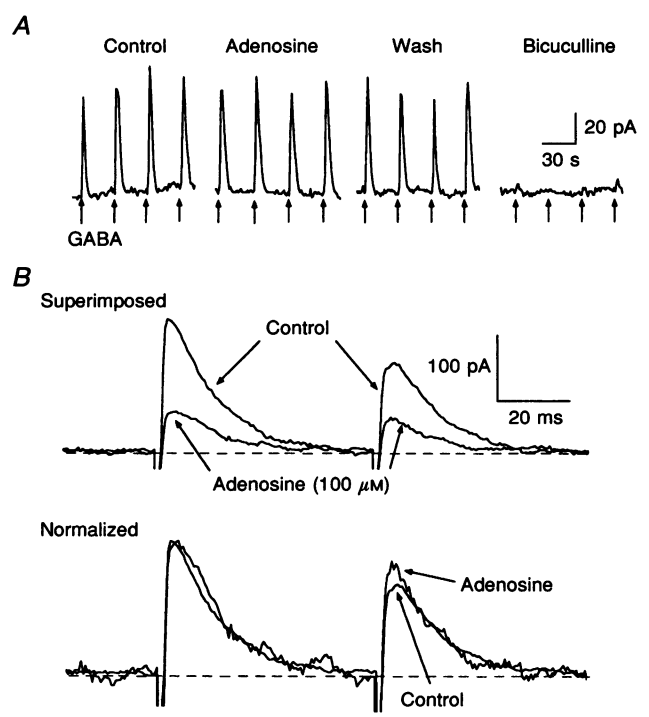

Figure 6. Adenosine reduces IPSC amplitude by presynaptic inhibition of GABA release

A, adenosine (100 μM) did not reduce outward currents evoked by exogenous GABA. The upward arrows indicate the times at which GABA was ejected by pressure from a micropipette. Outward currents are completely blocked by bicuculline (30 μM). *B*, upper traces show superimposed IPSCs evoked by pairs of stimuli recorded in the presence and absence of adenosine (100 μM). Without adenosine (control), the second IPSC is smaller than the first. However, in the presence of adenosine, the second IPSC is nearly the same size as the first. The lower traces are the same as those above, except that the amplitude of the first IPSCs recorded in adenosine has been normalized to match the first control IPSC. Paired stimuli were delivered 50 ms apart.

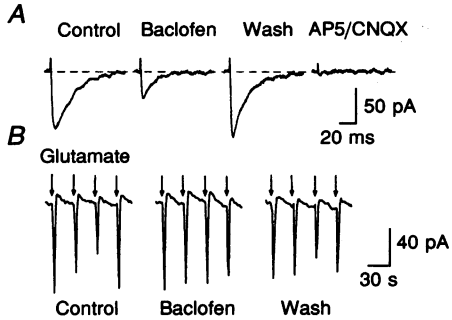

Figure 7. Effects of baclofen on glutamate-mediated EPSCs in SNR neurones

A, current traces show that baclofen (1 μM) reversibly reduces EPSC amplitude. This EPSC was recorded in bicuculline (30 μM) and was abolished by a combination of AP5 (50 μM) and CNQX (10 μM). The interrupted line indicates zero holding current. *B*, baclofen (1 μM) fails to reduce inward currents evoked by glutamate applied locally to the slice by pressure-ejection from a micropipette. Glutamate applications are indicated by the downward arrows.

In order to identify the site of action of adenosine, outward currents were evoked by GABA that was ejected by pressure from tips of micropipettes placed near the recording pipette. Adenosine (0·1–1 mM) had no effect on currents evoked by local application of GABA ($n = 5$; Fig. 6*A*). These GABA-induced currents were mediated by $GABA_A$ receptors because they were completely blocked by bicuculline (30 μM). In a second series of experiments, effects of adenosine were studied on amplitudes of $GABA_A$ IPSCs evoked by a pair of stimuli delivered 50 ms apart. Under control conditions, the ratio of the amplitude of the second IPSC divided by the first IPSC was 0·70 ± 0·03 ($n = 3$). As seen in Fig. 6*B*, adenosine (100 μM) significantly increased the IPSC paired-pulse ratio to 0·85 ± 0·01 ($n = 3$) ($P < 0·01$, paired t test). These data support the conclusion that adenosine acts presynaptically to inhibit GABA release.

Studies on glutamate-mediated EPSCs

Baclofen inhibits EPSCs. Recording in the presence of bicuculline (30 μM), a single stimulus evoked an EPSC with an average amplitude of 185 ± 20 pA (range 54–598 pA, $n = 30$). As seen in Fig. 7*A*, baclofen (1 μM) reversibly reduced the EPSC. EPSCs were mediated by glutamate because they were completely abolished by perfusion with AP5 (50 μM) and CNQX (10 μM) (Fig. 7*A*). As seen in Fig. 7*B*, transient inward currents evoked by glutamate were not reduced by baclofen (1 μM). In these experiments, glutamate was applied locally to the slice by pressure-ejection from tips of micropipettes. Thus baclofen does not inhibit EPSCs by a postsynaptic site of action. It is most likely that baclofen acts presynaptically to inhibit glutamate release from nerve terminals.

Baclofen-induced inhibition of EPSCs was concentration dependent with an IC_{50} of 0·78 ± 0·13 μM ($n = 7$) (Fig. 8*A*); this IC_{50} is not significantly different from the IC_{50} for baclofen-induced inhibition of $GABA_A$ IPSCs ($P = 0·12$; t test). The highest concentration of baclofen tested (10 μM) reduced EPSCs by 80 ± 3% ($n = 7$). Increasing concentrations of CGP 35348 (30–300 μM) progressively

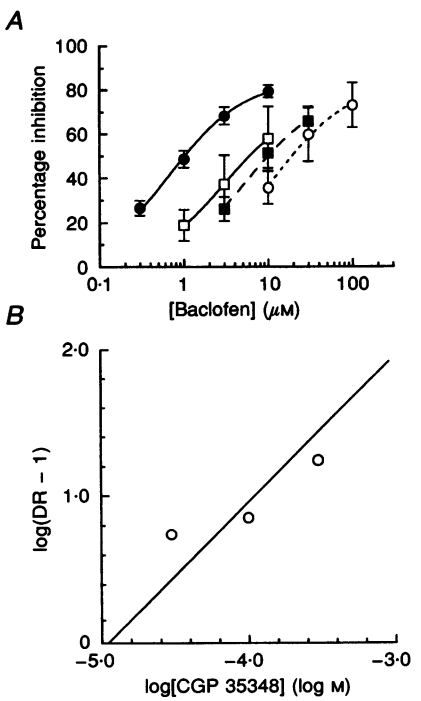

Figure 8. CGP 35348 antagonizes the ability of baclofen to inhibit EPSCs

A, baclofen concentration–response curves for percentage inhibition of EPSCs in the absence and presence of different concentrations of CGP 35348 (●, zero; □, 30 μM; ■, 100 μM; ○, 300 μM). Without CGP 35348, the baclofen IC_{50} is 0·78 ± 0·13 μM ($n = 7$). Increasing concentrations of CGP 35348 progressively shift the baclofen concentration–response curve to the right. *B*, Schild plot from data in *A* yields a K_d of 11 μM for CGP 35348.

shifted the baclofen concentration–response curve to the right, as seen in Fig. 8*A*. The baclofen IC_{50} was 5·1 ± 3·6 μM in 30 μM CGP 35348 ($n = 3$), 6·4 ± 3·7 μM in 100 μM CGP 35348 ($n = 4$), and was 14·5 ± 5·9 μM in 300 μM CGP 35348 ($n = 3$). Using results from the Schild plot analysis shown in Fig. 8*B*, the K_d for CGP 35348 was estimated to be 11 μM. Thus the K_d of CGP 35348 estimated for baclofen-induced inhibition of EPSCs was similar to the K_d of CGP 35348 derived for baclofen-induced inhibition of IPSCs (5 μM).

Adenosine also inhibits EPSCs. As shown in Fig. 9*A*, adenosine (100 μM) and CPA (0·1 μM) reversibly reduced the EPSC amplitude by about 70%, and these effects were completely blocked by DPCPX (1 μM). Furthermore, Fig. 9*B* shows that effects were concentration dependent; the IC_{50} for adenosine was 57 ± 19 μM ($n = 5$), whereas the IC_{50} for CPA was 0·034 ± 0·0010 μM ($n = 3$). Neither of these IC_{50} values were significantly different from those for inhibition of $GABA_A$ IPSCs by adenosine and CPA ($P > 0·15$, *t* tests). Because adenosine (100 μM) did not reduce inward currents evoked by exogenous glutamate, which was applied locally by pressure-ejection from micropipettes ($n = 4$; Fig. 9*C*), the reduction of EPSCs by adenosine is not due to a postsynaptic site of action. Thus these data suggest that adenosine acts at presynaptic A_1 receptors to inhibit glutamate release.

DISCUSSION

These studies show that baclofen and adenosine reduce both glutamate EPSCs and $GABA_A$ IPSCs by more than 80%. The inhibitory actions of these agents were not mediated by a postsynaptic site because adenosine and baclofen did not block currents evoked by local application of glutamate or GABA. Furthermore, using pairs of pulses to evoke $GABA_A$ IPSCs, we found that baclofen and adenosine significantly increased the amplitude of the second GABA IPSC with respect to the first one. This observation is consistent with the possibility that baclofen and adenosine reduce the amount of synaptically released GABA that would ordinarily be available to transiently inhibit further GABA

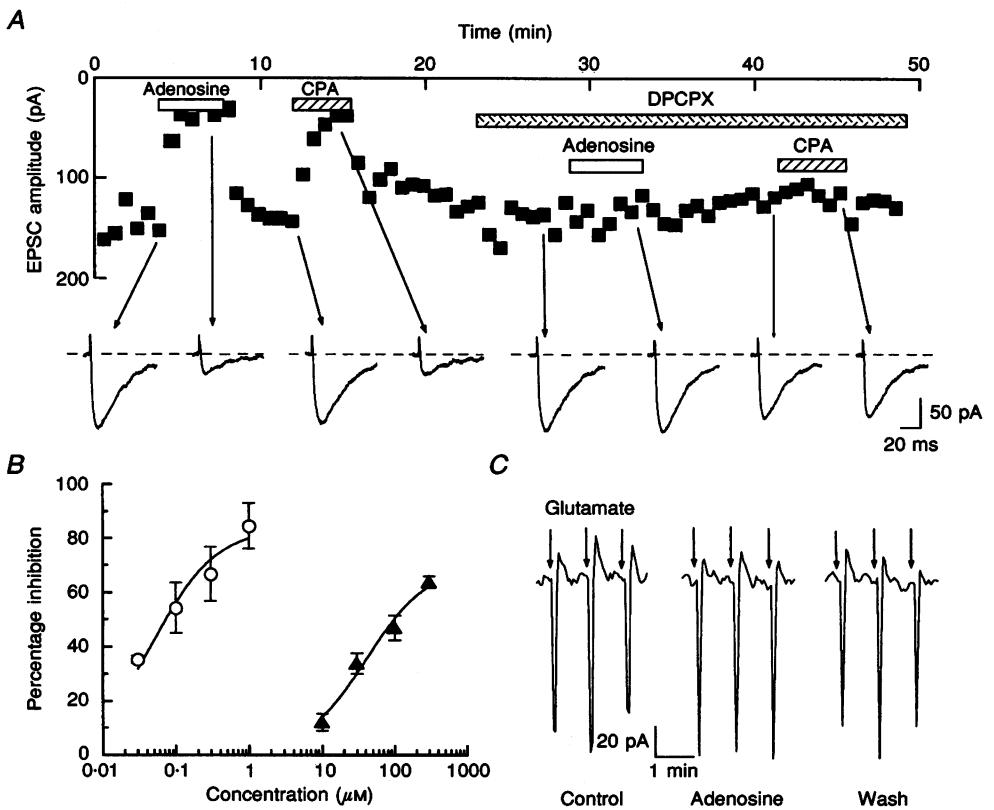

Figure 9. Adenosine inhibits glutamate-mediated EPSCs

A, both adenosine (100 μM) and CPA (0·1 μM) reversibly reduce the EPSC amplitude in this SNR neurone. Furthermore, perfusion with DPCPX (1 μM) completely blocks effects of adenosine and CPA. EPSCs were evoked every 40 s; representative EPSCs are shown at the times indicated by the arrows. *B*, adenosine (▲) and CPA (○) concentration–response curves for inhibition of EPSCs. The IC_{50} for adenosine was 57·4 ± 19·4 μM ($n = 5$), whereas the IC_{50} for CPA was 0·034 ± 0·010 μM ($n = 3$). *C*, adenosine (100 μM) fails to reduce inward currents evoked by exogenous glutamate ejected by pressure from a micropipette. The downward arrows indicate applications of glutamate. These results show that inhibition of EPSCs by adenosine is not mediated by a postsynaptic site of action.

release (Davies *et al.* 1990), but mechanisms other than autoinhibition by GABA are also possible. The fact that CGP 35348 blocks effects of baclofen shows that baclofen acts via $GABA_B$ receptors. Similarly, our findings that adenosine is blocked by the A_1 antagonist DPCPX and is most potently mimicked by A_1 agonists suggest that adenosine acts via A_1 receptors. Taken together, our results suggest that adenosine and baclofen stimulate presynaptic A_1 and $GABA_B$ receptors, which reduce the release of glutamate and GABA from nerve terminals in the SNR.

We found that baclofen, but not adenosine, evoked a small outward current (about 15 pA) in about 40% of all SNR neurones. This weak response, which only occurred at the highest concentration of baclofen tested (10 μM), was similar to that reported previously by Lacey and co-workers (Rick, Stanford & Lacey, 1995; Stanford & Lacey, 1996). The small baclofen-induced outward current is in marked contrast to that evoked in presumed dopamine-containing neurones. Although this weak effect of baclofen could be due to a low level of expression of $GABA_B$ receptors or to inefficient coupling of a second messenger system to ion channels, this current could also have been produced 'indirectly' by an inhibition of the tonic release of excitatory neurotransmitters in the slice.

Reticulata neurones send collateral axons to the SNC to synapse onto dendrites of dopamine neurones. These synaptic contacts form the anatomical basis by which hyperpolarization of a SNR neurone can disinhibit a dopamine neurone and thereby increase dopamine release from nerve terminals (Grace & Bunney, 1979; Holstein, Pasik & Hámori, 1986). Although the action of opiates is one of the best-characterized examples of disinhibition of dopamine neurones, the findings by us and others (Rick *et al.* 1995) that [Met^5]enkephalin has no effect on membrane current in reticulata cells fail to support the idea that opioids disinhibit dopamine neurones by an action in the SNR. In contrast, previous work by North and co-workers showed that the disinhibition of dopamine neurones by opiates is mediated by GABA-containing interneurones in the midbrain that are hyperpolarized by μ-opioid agonists (Lacey, Mercuri & North, 1989; Johnson & North, 1992). These data suggest that the GABA interneurone in the SNC is distinctly different from the main GABA-containing output neurone in the SNR, although both types of cell inhibit dopamine neurones by activation of $GABA_A$ receptors (Johnson & North, 1992; Tepper, Martin & Anderson, 1995).

Based upon differences in affinities for agonists and antagonists, several studies have presented evidence for at least three different subtypes of $GABA_B$ receptor (Bonanno & Raiteri, 1993*a*), although at this time only one $GABA_B$ receptor (with two splice variants) has been cloned (Kaupmann *et al.* 1997). In rat cerebral cortex, $GABA_B$ receptors that inhibit [^{3}H]GABA release are reported to have higher affinity for some $GABA_B$ receptor antagonists than those $GABA_B$ receptors that inhibit glutamate release (Lanza, Fassio, Gemignani, Bonanno & Raiteri, 1993; Bonanno, Fassio, Schmid, Severi, Sala & Raiteri, 1997). One study, however, could not confirm this finding (Waldmeier, Wicki, Feldtrauer, Mickel, Bittiger & Baumann, 1994). Other studies in cerebral cortex have shown that inhibition of [^{3}H]GABA release by baclofen was not blocked by CGP 35348, whereas release was insensitive to baclofen and yet sensitive to CGP 35348 in spinal cord (Fassio, Bonanno, Cavazzani & Raiteri, 1994). In our study, the K_d values estimated for antagonism of baclofen-induced inhibition of $GABA_A$ IPSCs (5 μM) and glutamate EPSCs (11 μM) by CGP 35348 were close to the IC_{50} value of 14 μM which was obtained in hippocampal slices for block of $GABA_B$ receptor-mediated EPSCs (Solís & Nicoll, 1992), and the value of 4 μM, which was obtained in slices of rat cerebral cortex for antagonism of baclofen-induced inhibition of electrically evoked [^{3}H]GABA release (Waldmeier *et al.* 1994). On the other hand, our estimates of K_d are lower than that obtained by Bon & Galvan (1996) for inhibition of baclofen-induced hyperpolarization of dorsolateral septal neurones (32 μM), but they are higher than the value of 1·1 μM obtained for antagonism of baclofen-induced inhibition of [^{3}H]GABA release from slices of rat spinal cord (Bonanno & Raiteri, 1993*b*). Our data do not support the conclusion that release of GABA and glutamate are mediated by different subtypes of presynaptic $GABA_B$ receptor in the SNR.

There is considerable evidence that increased output from SNR neurones contributes to the rigidity and bradykinesia of Parkinson's disease (Robertson, 1992; Double & Crocker, 1995), and may also facilitate the generalization and spread of seizure discharges in epilepsy (Gale, 1985). In contrast, reduced output from the SNR has been linked to the hyperkinetic orofacial movements of tardive dyskinesia (DeLong, 1990; Mitchell, Crossman, Liminga, Andren & Gunne, 1992). Selective modulation of GABA or glutamate release in the SNR could be useful in the treatment of these disorders. For example, presynaptic inhibition of glutamate release from the subthalamonigral pathway would be expected to reduce the output from SNR neurones and thereby improve symptoms in patients with Parkinson's disease (Chesselet & Delfs, 1997). In tardive dyskinesia, presynaptic inhibition of GABA release would increase SNR neuronal activity which should reduce dyskinesia (Shirakawa & Tamminga, 1994). *In vivo*, synaptic inputs may be able to control the release of GABA such that presynaptic inhibition does not occur simultaneously at glutamate and GABA nerve terminals. Synaptic inputs could also selectively release adenosine (Yamamoto, Staines, Dewar, Geiger, Daddona & Nagy, 1988), although it is more likely to be released diffusely (Mitchell, Lupica & Dunwiddie, 1993). However, in order for an adenosine or GABA agonist to have a clinically useful action in the SNR, it must somehow be selective for either the glutamate- or GABA-containing nerve terminal, because inhibition of both glutamate and GABA release on the same neurone would be of questionable use.

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Acknowledgements

This work was supported by a grant from the USPHS (ROI NS35437).

Author's email address

S. W. Johnson: johnsost@ohsu.edu

Received 28 May 1997; accepted 23 July 1997.