Journal of Physiology (1988), **401**, *pp.* 437–453
With 7 text-figures
Printed in Great Britain

ON THE POTASSIUM CONDUCTANCE INCREASE ACTIVATED BY $GABA_B$ AND DOPAMINE D_2 RECEPTORS IN RAT SUBSTANTIA NIGRA NEURONES

BY M. G. LACEY, N. B. MERCURI* AND R. A. NORTH

From the Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201, U.S.A.

(*Received 12 May 1987*)

SUMMARY

1. Intracellular recordings were made from 193 substantia nigra zona compacta neurones in slices of rat mesencephalon. All cells were hyperpolarized by baclofen; this was accompanied by a fall in input resistance. Cells voltage clamped at −60 mV showed an outward current associated with a conductance increase in response to baclofen. The baclofen effects were concentration dependent (effective range 0·3–30 μM); the concentration producing half the maximal effect was 1·5 μM. (−)-Baclofen was 300–700 times more potent than (+)-baclofen.

2. The potential change or membrane current caused by baclofen reversed polarity at $-108{\cdot}8 \pm 1{\cdot}1$ mV ($n = 10$) when the potassium ion concentration was 2·5 mM, $-96{\cdot}0 \pm 2{\cdot}8$ mV ($n = 3$) in 4·5 mM-potassium and $-76{\cdot}6 \pm 1{\cdot}7$ mV ($n = 5$) in 10·5 mM-potassium. The relationship between reversal potential and potassium concentration conformed to the Nernst equation.

3. Dopamine was also applied to 119 of these neurones; all exhibited either a hyperpolarization or an outward current.

4. Baclofen and dopamine outward currents were reduced reversibly by barium (100–300 μM) and tetraethylammonium (10 mM). Superfusion for 5–10 min with solutions presumed to block calcium currents reduced, but did not abolish, responses to baclofen. The effect of baclofen persisted in tetrodotoxin (1 μM).

5. Superfusion of γ-aminobutyric acid (GABA, 0·3–3 mM) caused either membrane depolarization or hyperpolarization, accompanied by a fall in input resistance. The depolarization was mimicked by muscimol (10 μM) and blocked by bicuculline methiodide (10–100 μM); the hyperpolarization was resistant to bicuculline. Nipecotic acid (500 μM) enhanced the effect of GABA, but was without effect upon the actions of muscimol and baclofen.

6. The effect of dopamine was enhanced by cocaine (10 μM) and antagonized by (−)-sulpiride (0·1–1 μM), whereas the actions of baclofen were unaffected by cocaine or (−)-sulpiride. The maximum outward current produced by dopamine was approximately half that produced by baclofen.

* Present address: Clinica Neurologica, Il Universita Degli Studi de Roma, Via Orazio Raimondo, 00173 Roma, Italy.

7. Outward currents produced by dopamine were reversibly occluded by maximal outward currents caused by baclofen.

8. Baclofen and dopamine hyperpolarizations were unaffected by intracerebroventricular injection of animals with pertussis toxin.

9. Cells impaled with electrodes containing guanosine 5′-*O*-(3-thiotriphosphate) (1 mM) were hyperpolarized by both baclofen and dopamine, but the membrane potential did not fully return to its original level when agonist application was discontinued.

10. It is concluded that activation of both dopamine D_2 and $GABA_B$ receptors may increase the same potassium conductance. The receptors are coupled to the potassium channels by a guanosine 5′-triphosphate (GTP)-binding protein.

INTRODUCTION

The existence of a striato/pallido-nigral pathway containing γ-aminobutyric acid (GABA) is well established by both anatomical and electrophysiological studies (for reviews see Fonnum & Storm-Mathisen, 1978; Dray, 1979). Synaptic contacts between GABA-containing nerve terminals and dopamine-containing neurones of the substantia nigra zona compacta have been demonstrated (Van den Pol, Smith & Powell, 1985). Mediation by GABA of synaptic transmission in the striato-nigral pathway is considered to exert a negative feed-back influence on nigral dopamine neurones (Carlsson & Lundqvist, 1963), which in turn project to the striatum and have been linked for some time with the control of voluntary movement.

It has been found that GABA inhibits the firing of rat substantia nigra compacta neurones and this effect has been described as being both sensitive (Olpe, Koelle, Wolf & Haas, 1977) and insensitive (Pinnock, 1984) to the 'classical' GABA antagonist bicuculline. It is known that GABA binds to two distinct sites in mammalian nervous tissue, termed $GABA_A$ and $GABA_B$ receptors by Hill & Bowery (1981). Binding of GABA to the $GABA_B$ site is unaffected by bicuculline, but is competitively and stereoselectively displaced by baclofen (Hill & Bowery, 1981). Baclofen also inhibits firing in nigral compacta neurones (Olpe *et al.* 1977) and Pinnock (1984) has shown that it causes a hyperpolarization.

In the present study, intracellular electrophysiological recordings from a rat brain slice preparation were used to establish the ionic mechanism of the action of baclofen in the neurones of the substantia nigra zona compacta. The potassium conductance increased by baclofen was compared to that increased by dopamine on these cells (Lacey, Mercuri & North, 1987) and the mechanism coupling $GABA_B$ and D_2 receptors to that conductance has been investigated.

METHODS

The experimental preparation

The preparation and *in vitro* maintenance of slices of rat brain containing the substantia nigra compacta has been described previously (Lacey *et al.* 1987). Briefly, a block of tissue including the mesencephalon was prepared from the brain of a male Sprague–Dawley rat and serial coronal slices (thickness 300 μm) were cut on a Vibratome (Oxford). A single brain slice containing the substantia nigra was transferred to a recording chamber, wherein it was totally immersed in a

solution continuously flowing at a rate of 1·5 ml/min and maintained at 36 °C. The solution contained (mM): NaCl, 126; KCl, 2·5; NaH_2PO_4, 1·2; $MgCl_2$, 1·3; $CaCl_2$, 2·4; glucose, 10; $NaHCO_3$, 26; it was saturated with 95% O_2 and 5% CO_2 at 36 °C. When $CoCl_2$ was added to the solution, NaH_2PO_4 was omitted.

Electrical recording techniques

Intracellular recordings were made from the substantia nigra zona compacta using glass microelectrodes as described previously (Lacey *et al.* 1987). Electrodes were filled with 2 M-KCl and, in some experiments, also with guanosine 5′-*O*-(3-thiotriphosphate) (GTP-γ-S; Boehringer Mannheim; 1, 2 or 20 mM). Recordings of membrane potential were amplified with a WPI M707 amplifier: either a Dagan 8100 or Axoclamp 2 single-electrode voltage-clamp amplifier was also used for both current- and voltage-clamp recordings. Voltage-clamp recordings of membrane currents were made using a 2–3 kHz switching frequency and either a 25 or 33% duty cycle. Membrane potentials and currents were recorded on Gould 2400 chart recorders. Steady-state current–voltage plots were obtained by hyperpolarizing neurones to −110 to −130 mV and then depolarizing at a steady rate of 1 mV/s; membrane current was continuously plotted as a function of membrane potential using an *X–Y* plotter (Houston Instruments 200).

Application of substances

(*p*-Chlorophenyl)-γ-butyric acid (baclofen; Lioresal; Ciba–Geigy) was applied by changing the superfusion solution to one containing a known concentration of baclofen. Solutions containing baclofen entered the recording chamber within 30 s of turning a tap, the delay being necessary for passage of the solution through a heat exchanger. Complete exchange of the bath solution occurred within 2·5 min, during which time the effect of superfused baclofen had reached a maximum. Baclofen (1 mM) was also applied by pressure ejection from micropipettes with their tips (5–20 μM diameter) positioned in the superfusing solution above the slice surface. Reproducible responses to ejection of baclofen were achieved using pressure pulses of 50–1000 ms duration (Picospritzer II, General Valve Corporation). Reproducible responses to baclofen were also obtained using the 'fast tap' technique by which a high concentration of baclofen (30–100 μM) was superfused for 10–20 s (a period insufficient to attain equilibrium in the slice chamber). Stock solutions of dopamine hydrochloride (Sigma) were kept on ice and gassed with N_2; they were applied either by superfusion in known concentrations or by pressure ejection in the same manner as described above for baclofen. The following drugs were also applied by superfusion: (+)- and (−)-baclofen hydrochloride (Ciba–Geigy), (±)-γ-aminobutyric acid (GABA, Sigma), muscimol (Sigma), (−)-bicuculline methiodide (Sigma), (±)-nipecotic acid (Sigma), cocaine hydrochloride (Sigma) and (−)-sulpiride (gift from Dr Forgione, Ravizza, Milan). Pre-treatment of rats with an intracerebroventricular injection of pertussis toxin (List; 1·5 μg in 15 μl vehicle under sodium thiopentone (65 mg/kg) anaesthesia) was performed as described by Aghajanian & Wang (1986) and Andrade, Malenka & Nicoll (1986). Recordings were made from brain slices taken from these rats 3–4 days after treatment.

Numerical data are expressed as mean ± standard error of the mean.

RESULTS

Baclofen actions were studied on 193 neurones located in the substantia nigra zona compacta from which intracellular recordings were made (durations 0·3–9 h). Of these neurones impaled with electrodes containing only 2 M-KCl, 145 fired action potentials spontaneously at regular frequencies ranging from 0·3 to 7·0 Hz (mean 2·3 ± 0·22 Hz, $n = 90$) after 15 min of microelectrode impalement. Firing of action potentials could be prevented by the passage of constant hyperpolarizing current of 50–150 pA, demonstrating the threshold for action potential generation to be −58·9 ± 0·8 mV (fifty-nine cells). The remaining thirty-six neurones were quiescent, but could fire action potentials in response to depolarizing current injection. These cells had resting membrane potentials of −61·1 ± 1·5 mV ($n = 36$). Of these neurones,

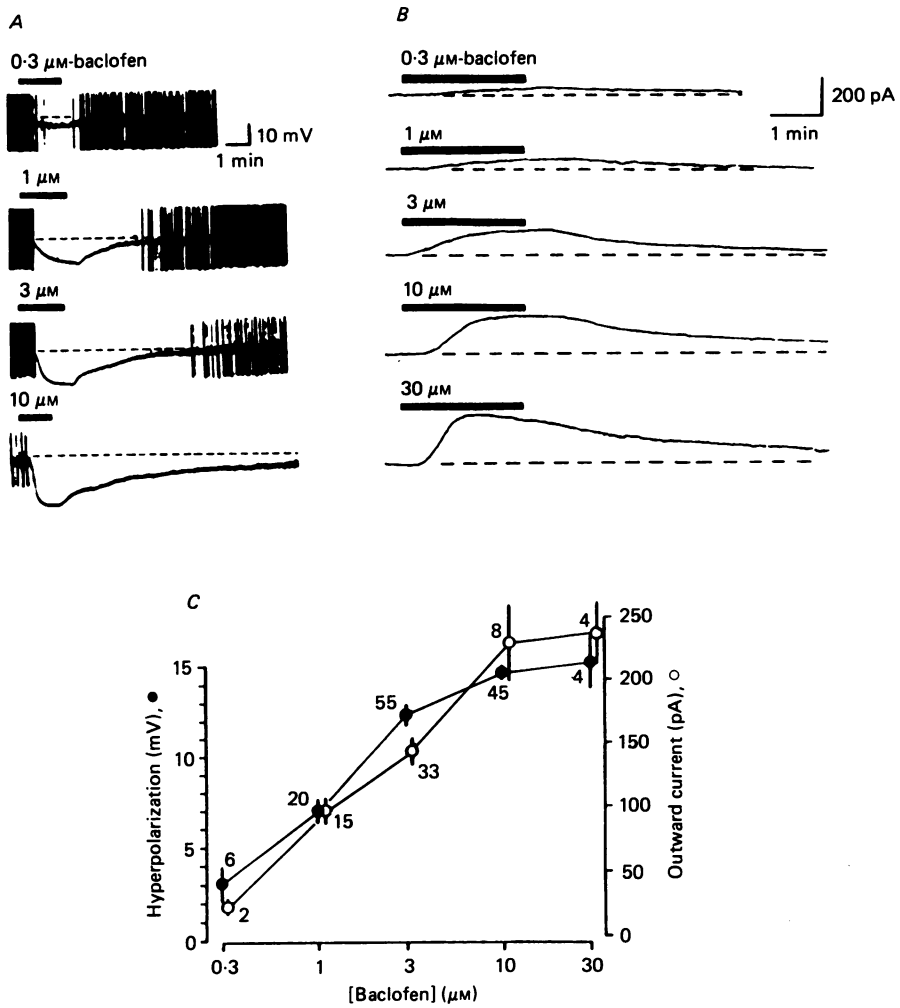

Fig. 1. Baclofen (0·3–30 μM) inhibited cell firing, hyperpolarized the membrane and caused an outward current in a concentration-dependent manner. *A*, records of membrane potential from a single cell at resting potential firing spontaneous action potentials (full amplitude not reproduced). Baclofen, applied in the superfusate at the concentrations indicated for the periods denoted by the bars (which include the 30 s dead time of the superfusion system), prevented cell firing. This was accompanied by a hyperpolarization whose magnitude was dependent upon the concentration of baclofen applied. Dashed line indicates −55 mV. *B*, records of membrane current from another cell with membrane potential clamped at −60 mV. Baclofen produced an outward current, the amplitude of which was concentration dependent. Dashed line indicates holding current level of −60 pA. *C*, effects of baclofen as a function of concentration applied. ●, membrane hyperpolarization; ○, outward current (at holding potentials of −55 to −70 mV). Points show mean effect and vertical bars the S.E.M. for number of neurones indicated.

119 were also tested with dopamine (1–300 μM). In all cases dopamine caused a reversible reduction in firing rate, a membrane hyperpolarization or outward current as described previously (Lacey *et al.* 1987).

The action of baclofen

Baclofen (0·3–30 μM) stopped action potential firing and hyperpolarized the membrane by up to 26 mV. This effect was dependent upon the concentration of baclofen added to the superfusate (Fig. 1*A*) and was qualitatively similar to

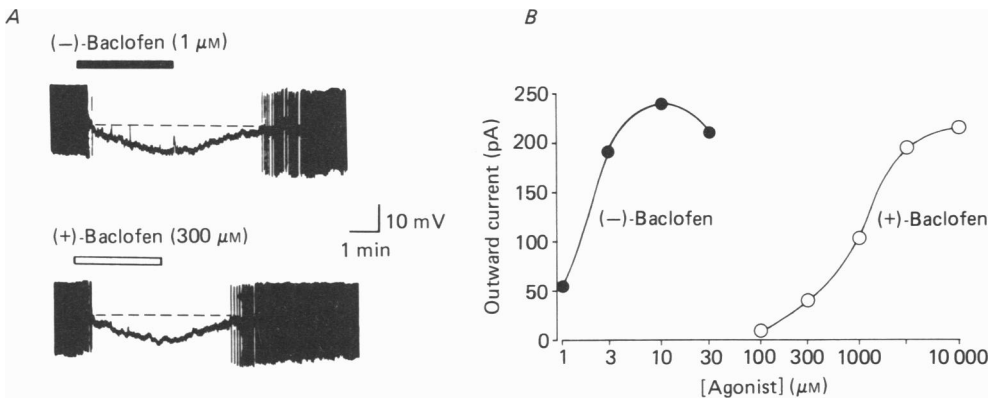

Fig. 2. The effect of baclofen was stereoselective. *A*, records of membrane potential from the same cell which was firing spontaneous action potentials. Inhibitions of firing and hyperpolarizations of roughly equal magnitude were caused by superfusions of (−)-baclofen (1 μM; filled bar, upper trace) and (+)-baclofen (300 μM; open bar, lower trace). Dashed line indicates −57 mV. *B*, concentration–response curve from another cell showing outward currents produced by superfusion of (−)-baclofen (●) and (+)-baclofen (○). The half-maximal response was about 125 pA; the concentrations of (−)- and (+)-baclofen required for this effect were 1·8 μM and 1·2 mM respectively: a potency ratio of 666.

responses to either the pressure ejection or 'fast tap' method of drug application. Hyperpolarizations evoked by baclofen were reproducible upon repeated applications of the same quantity of the drug whether it was superfused, pressure-ejected or applied by the 'fast tap' method. The hyperpolarizing action of baclofen was accompanied by a fall in input resistance. Under voltage clamp, at holding potentials of between −55 and −70 mV, baclofen produced an outward membrane current which was also dependent upon the concentration applied (Fig. 1*B*).

Responses to superfused baclofen took 2–3 min to reach a steady state (Fig. 1) and, following wash-out of baclofen with drug-free solution, at least a further 5 min for membrane potential, current or firing rate to recover its control level; this period was longer for higher concentrations of baclofen (Fig. 1). Recovery from hyperpolarizations greater than 15 mV was often incomplete even after wash-out periods of up to 30 min. Reversal of the effects of the more rapid methods of baclofen application occurred faster, but with large effects it was still sometimes incomplete.

The relationship between baclofen concentration and effect was the same whether

hyperpolarization or outward current was measured. The concentration of baclofen required to produce 50 % of its maximal hyperpolarizing effect (IC_{50}) was 1·3 μM, and 1·7 μM was needed for the half-maximal outward current (Fig. 1*C*). Near-maximal responses to baclofen (at concentrations of 10–30 μM) declined in amplitude to approximately 80 % of their peak while baclofen was still present in the superfusate; the decline began within 90 s of the onset of baclofen application (see Figs 1*B* and 7). Increasing the concentration of baclofen in the superfusate could overcome the

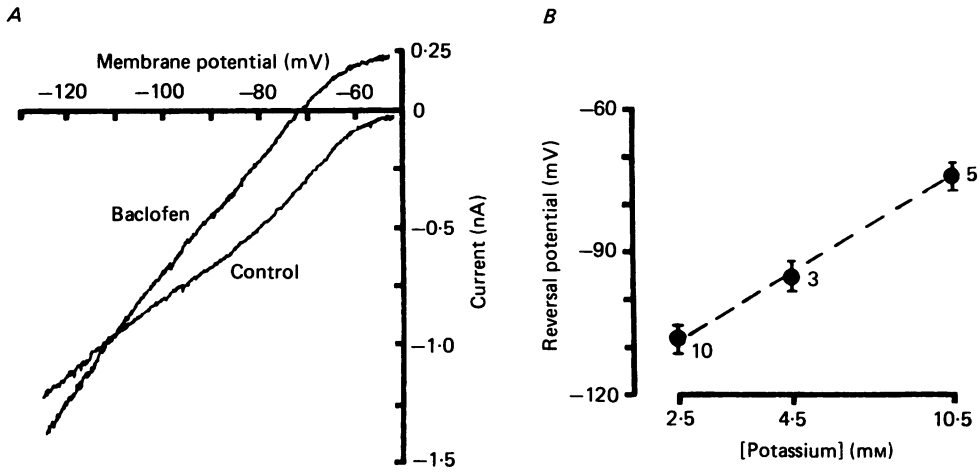

Fig. 3. Baclofen increases membrane conductance and has a reversal potential dependent upon extracellular potassium concentration. *A*, current–voltage relation before and during superfusion of baclofen. The two current–voltage plots were constructed by depolarizing the same neurone from −125 to −52 mV at a rate of 1 mV/s while measuring membrane current. Baclofen concentration was 10 μM. Baclofen current reversed from outward to inward at about −110 mV (potassium concentration was 2·5 mM); note the increase in conductance throughout the voltage range. *B*, reversal potential of baclofen response plotted as a function of the logarithm of the potassium ion concentration. Data pooled from twelve cells. Points are mean values and vertical bars are S.E.M., for the numbers of neurones indicated. Line, fitted by linear regression, has slope of 52 mV per log unit of potassium ion concentration.

decline in response amplitude only transiently. Hyperpolarizations and outward currents resulting from application of lower concentrations of baclofen (less than 10 μM) did not decline during applications of up to 10 min. Both enantiomers of baclofen caused hyperpolarizations and outward currents; (−)-baclofen was 300–700 times more potent than (+)-baclofen on the seven neurones tested (Fig. 2).

Membrane potential changes ($n = 4$) or membrane currents ($n = 1$) resulting from pressure application of baclofen were measured during conditioning membrane potential changes. The response to baclofen changed polarity, from hyperpolarization to depolarization, or from outward to inward current, as the membrane potential was made more negative than −100 mV. In five further experiments steady-state current–voltage plots were made in the presence and absence of baclofen in the superfusate. These plots similarly showed a reversal in polarity of baclofen-induced current at membrane potentials more negative than −100 mV (Fig. 3*A*). The

current–voltage plots also demonstrated that the baclofen outward current was accompanied by an increase in membrane conductance (Fig. 3*A*). The mean reversal potential of baclofen as obtained by these methods was $-108{\cdot}8 \pm 1{\cdot}1$ mV in ten cells.

Whereas the above experiments were carried out with a solution containing 2·5 mM-potassium ions, in 4·5 mM-potassium the reversal potential of baclofen was $-96{\cdot}0 \pm 2{\cdot}8$ mV (three cells) and in 10·5 mM-potassium it was $-76{\cdot}6 \pm 1{\cdot}7$ mV (five cells: three experiments in current clamp and two in voltage clamp gave the same result). The baclofen reversal potential was related to the logarithm of the potassium ion concentration as shown for the pooled results from twelve cells in Fig. 3*B*. The slope of the line relating reversal potential to the logarithm of the potassium concentration was 52 ± 7 (95% confidence limits). A slope of 60 is predicted by the Nernst equation for a selective increase in potassium conductance at 36 °C.

Barium (100 or 300 μM) reduced the baclofen outward current by 52 and 81% respectively (Fig. 4*A*). Responses to baclofen were less sensitive to tetraethylammonium (TEA); 10 mM reduced the outward current by 37 ± 7% (four cells) and 30 mM by 44 ± 7% (three cells, Fig. 4*B*).

Hyperpolarizations and outward currents in response to baclofen were observed in all eight neurones where baclofen was applied in the presence of tetrodotoxin (TTX, 1 μM). In four further neurones where comparisons of baclofen hyperpolarizations were made in the absence and presence of TTX, no effect of TTX was observed: the amplitude of the hyperpolarizations in TTX was $90 \pm 7{\cdot}7$% of the control amplitude. Several different solutions were used in an attempt to block calcium conductances and, by implication, transmitter release from surrounding cells. These were normal solution (calcium concentration 2·5 mM) with either additional magnesium (10 mM) or cobalt (0·5–2 mM), low (0·5 mM) calcium solution with magnesium or cobalt, and a solution that contained no added calcium but with [ethylene-bis(oxyethylene-nitrilo)]tetraacetic acid (EGTA, 0·5–1 mM). All of these solutions blocked evoked or spontaneous firing of action potentials within 2 min and often caused a depolarization. The effects of calcium-free or cobalt-containing solutions were usually not fully reversible when the period of their application exceeded 10 min; the cessation of cell firing produced by magnesium (10 mM) was usually unaccompanied by changes in membrane potential and reversed on wash-out.

Hyperpolarizations or outward currents elicited by baclofen were reduced by $24 \pm 6{\cdot}4$% (four cells) in calcium-free solutions containing EGTA (3–6 min application), but in none of these experiments did the control response to baclofen recover following wash-out of calcium-free solution. Cobalt (0·5–2 mM, for 5–10 min) reduced baclofen hyperpolarizations or outward currents by $54 \pm 13{\cdot}6$% in six cells; when the cobalt was washed out for 15–40 min the baclofen response recovered to three-quarters of its control amplitude in three of four cells tested. Magnesium (10 mM, 10–15 min) reduced baclofen hyperpolarizations by 40 ± 15% (three cells); partial recovery of the response to baclofen was observed in only one cell after 20 min washing with normal solution. In brief, various solutions which blocked spontaneous action potentials did not abolish the action of baclofen, implying that calcium entry into the neurone under study is probably not required for the potassium conductance increase.

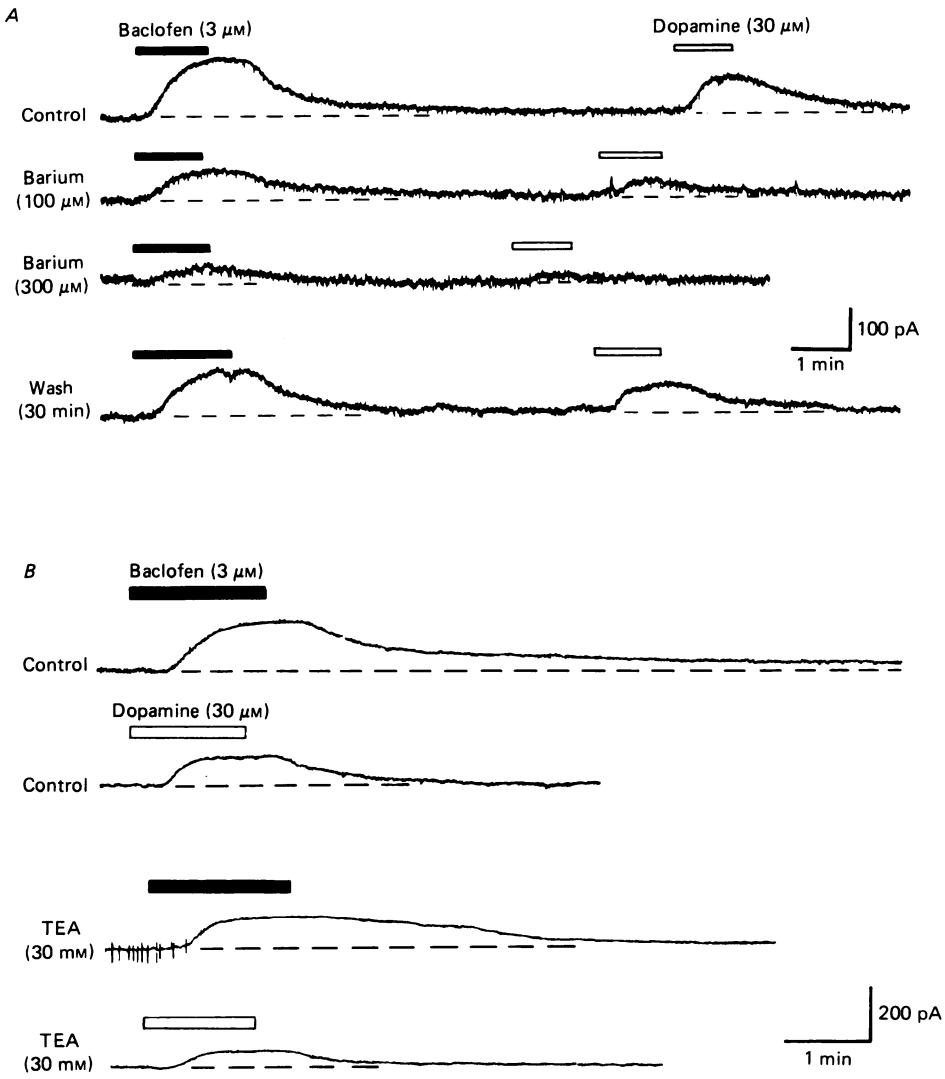

Fig. 4. Baclofen- and dopamine-induced outward currents show equal sensitivity to barium and high concentrations of tetraethylammonium (TEA). Records of membrane current from two different cells showing responses to superfusion of baclofen (filled bars) and dopamine (open bars). *A*, control outward currents in response to baclofen and dopamine (upper records) were reduced by barium (100 and 300 μM) to a similar degree (middle two records), partially recovering on wash-out (lower records). Membrane potential held at −62 mV. *B*, in a second cell, outward currents produced by baclofen and dopamine (upper two traces) were equally reduced by TEA (30 mM, lower two traces). Membrane potential held at −58 mV. Tetrodotoxin (1 μM) was present throughout.

The action of GABA and muscimol

γ-Aminobutyric acid (0·3–3 mM) was applied to twenty-five neurones. The predominant effect was a large fall in input resistance (reductions of up to 80% of control were seen in 3 mM-GABA). Either depolarization or hyperpolarization was also observed; in some cells GABA produced clearly biphasic effects, with the depolarizing component becoming more apparent at higher concentrations. At

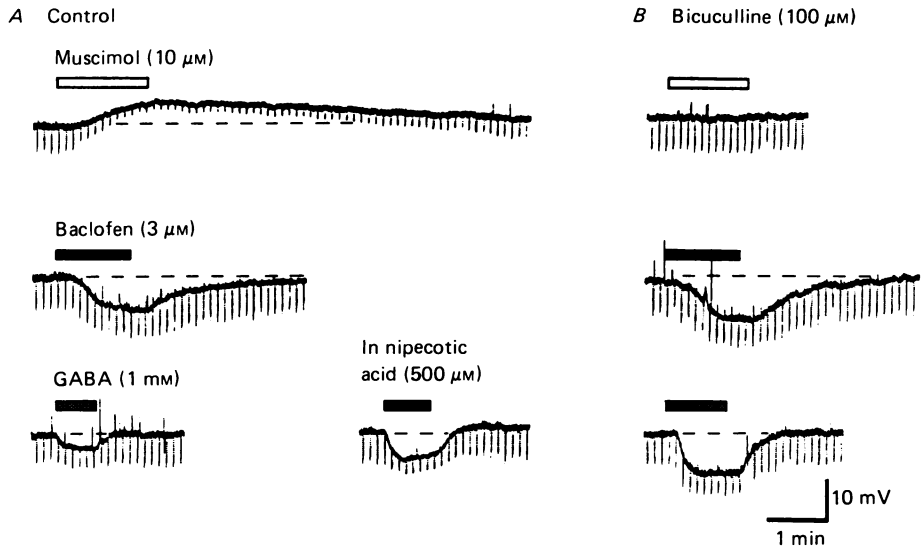

Fig. 5. Actions of muscimol, baclofen and GABA: antagonism of $GABA_A$ receptor-mediated depolarizations by bicuculline. Records of membrane potential from a single, non-firing neurone with resting potential −65 mV (dashed line). Transient downward deflections caused by passage of hyperpolarizing current pulses (50 pA, 1 s); their amplitude is proportional to input resistance. *A*, control. Responses to superfusion of $GABA_A$ receptor agonist muscimol (10 μM, open bars) and $GABA_B$ receptor agonist baclofen (3 μM, filled bars). Responses to GABA (1 mM, stippled bars) are shown both in the absence and presence of nipecotic acid (500 μM). *B*, in bicuculline (100 μM) and nipecotic acid (500 μM). Effects of the same three agonists are shown. The action of muscimol was blocked, that of baclofen little changed, whereas the hyperpolarization caused by GABA was increased.

300 μM-GABA, six of six cells were hyperpolarized (by 4·2±0·8 mV). At 1 mM-GABA, ten of fourteen cells were hyperpolarized (5·6±0·8 mV), two cells were depolarized (11·5 mV), one cell showed a biphasic response (hyperpolarization followed by depolarization) and one cell was unaffected. At 3 mM, four of nine cells were hyperpolarized (6·8±1·1 mV), three were depolarized (8·7±3·2 mV) and two showed biphasic responses. These effects all reversed readily when GABA application was discontinued.

Nipecotic acid blocks the uptake of [^{3}H]GABA and potentiates GABA-mediated depolarizations in brain slices when applied at a concentration of 1 mM (Brown, Collins & Galvan, 1980). In the present study, nipecotic acid (500 μM) increased the

amplitude of both hyperpolarizing and depolarizing components of the response to GABA in the three cells tested, as well as increasing the magnitude of the fall in input resistance caused by GABA (Fig. 5). Responses to baclofen were unaffected by nipecotic acid (Fig. 5). (−)-Bicuculline methiodide (10–100 μM), a selective $GABA_A$ receptor antagonist (Bowery, Hill, Hudson, Doble, Middlemiss, Shaw & Turnbull, 1980), reduced or blocked the depolarizing component of the response to GABA in all four cells tested and increased the amplitude of the hyperpolarization evoked by GABA: bicuculline also reduced the net fall in input resistance produced by GABA (Fig. 5). These actions of bicuculline were larger with higher concentrations. No effect of bicuculline was observed on the hyperpolarization induced by baclofen.

Muscimol, the $GABA_A$-selective agonist (Bowery, Hill & Hudson, 1983), caused a depolarization of $8{\cdot}8 \pm 1{\cdot}7$ mV (three cells, 10 μM) that was accompanied by a fall in input resistance (Fig. 5). Lower concentrations had smaller effects. The effect of muscimol took longer to reverse on wash-out than effects of comparable magnitude produced by GABA. This action of muscimol was insensitive to nipecotic acid, but was completely blocked by bicuculline (Fig. 5).

Comparison between the actions of baclofen and dopamine

Outward currents and hyperpolarizations produced by dopamine were potentiated by cocaine (10 μM), a known blocker of neuronal reuptake of catecholamines (Axelrod, 1971), and blocked by (−)-sulpiride, a competitive antagonist of dopamine D_2 receptors (Lacey *et al.* 1987). Baclofen outward currents and hyperpolarizations were insensitive to both these drugs and thus were not a result of either direct or indirect activation of dopamine D_2 receptors.

A striking difference between the effects of baclofen and dopamine was that baclofen appeared capable of producing larger hyperpolarizations and outward currents than dopamine. The maximum outward currents produced by dopamine in the presence of cocaine (10 μM) were measured in four cells; cocaine itself caused an outward current, presumably by blocking uptake of endogenous dopamine (Lacey *et al.* 1987), and the maximum outward current produced by dopamine was therefore taken to be the maximal effect of dopamine in cocaine plus the effect of cocaine alone. This value was 128 ± 17 pA (four cells). Comparison with the mean maximum baclofen outward current of about 235 pA (taken from Fig. 1*C*) indicated that on average the maximal effect of baclofen was approximately twice that of dopamine.

Interaction between baclofen and dopamine outward currents

Reproducible outward currents were evoked by pressure application or superfusion of dopamine ($110{\cdot}5 \pm 19$ pA in four neurones voltage clamped at $-61{\cdot}5 \pm 2{\cdot}3$ mV). In each of these cases, baclofen (1–30 μM) was then superfused for longer periods (up to 10 min) during which time the dopamine was reapplied. The concentration of superfused baclofen was then increased and the responses to dopamine elicited again. This procedure of prolonged cumulative application of baclofen combined with briefer test applications of dopamine was repeated until increasing the concentration of baclofen produced no further increase in the outward current. The effect of baclofen was then reversed by washing it from the tissue bath and the response to dopamine examined once more (see Fig. 6). The maximum outward current produced

by baclofen was 228 ± 24 pA ($n = 4$) and was attained with either 10 or 30 μM-baclofen. When the baclofen-induced outward current was submaximal, application of dopamine increased the net outward current (Fig. 6), but dopamine did not increase the net outward current to a value greater than the maximal baclofen current in any of the four cells tested. When the baclofen-induced current was maximal, dopamine had no further effect. After the baclofen outward current declined to zero during superfusion with drug-free solution, dopamine again produced outward currents similar to those in the control period (Fig. 6).

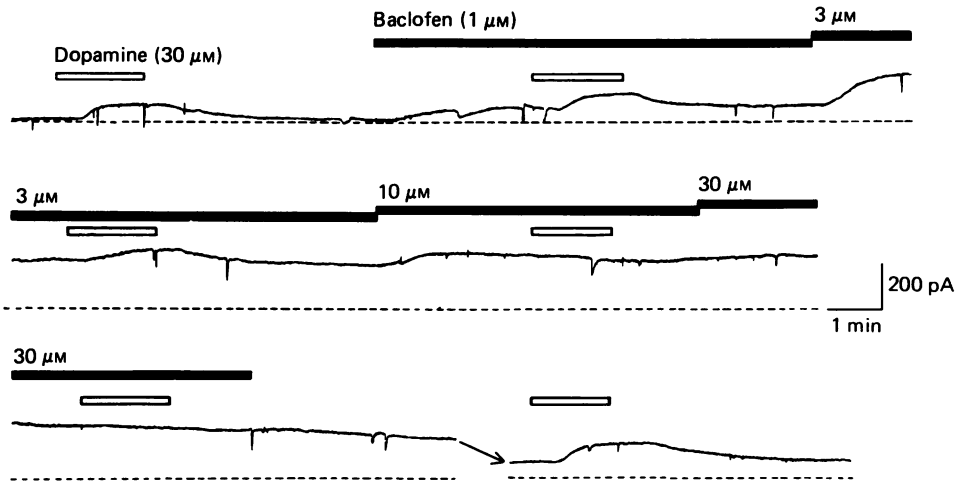

Fig. 6. Occlusion of the dopamine outward current occurs when the effect of baclofen is maximal. Continuous record of membrane current from a cell voltage clamped at -66 mV (except for a break of 20 min at the bottom arrow). Initial holding current was -80 pA (dashed line). Dopamine (30 μM, open bars) was superfused for 1·5 min every 6–8 min throughout the experiment to produce a reversible outward current (initial amplitude 70 pA). (−)-Baclofen was continuously superfused in increasing concentrations (1–30 μM, filled bars) and dopamine (30 μM) was applied again in the presence of each concentration of baclofen. Dopamine produced outward currents that were additive to those caused by baclofen; however, dopamine was unable to increase the net current to more than the maximum value produced by baclofen alone; thus in the presence of the maximal outward current produced by baclofen (10 and 30 μM) dopamine was without effect. Following reversal of the baclofen outward current by superfusion with drug-free solution, dopamine (30 μM) again produced an outward current equivalent to that seen before occlusion by baclofen.

Effects of intracellular GTP-γ-S

The effects of application of dopamine (30 μM) and baclofen (3 μM) by superfusion were examined upon twelve cells impaled with microelectrodes containing the non-hydrolysable GTP analogue GTP-γ-S. The electrical behaviour of the cells and the effects of dopamine and baclofen broadly fell into three categories, corresponding to the different concentrations of GTP-γ-S (1, 2 and 20 mM) used in the microelectrode.

None of the four cells impaled with electrodes containing 20 mM-GTP-γ-S fired

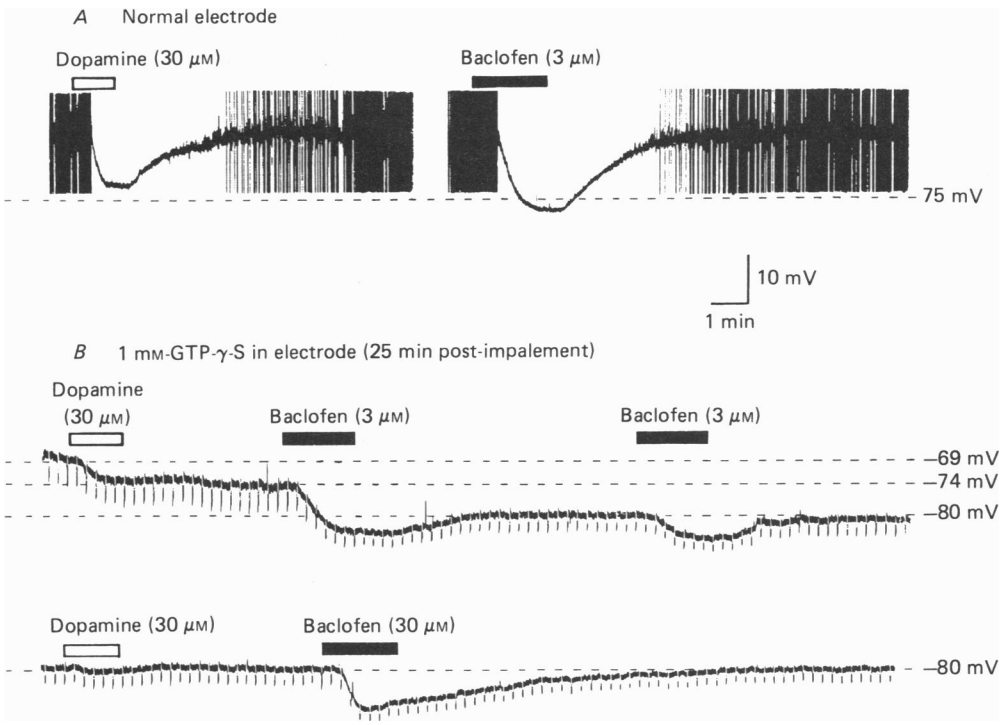

Fig. 7. Intracellular GTP-γ-S renders baclofen and dopamine hyperpolarization partially irreversible on wash-out of the agonist. Recordings from two different cells showing membrane potential and responses to superfused dopamine (open bars) and baclofen (filled bars). *A*, example of recording with normal 2 M-KCl-filled electrode. The cell fired spontaneous action potentials which were reversibly inhibited, accompanied by hyperpolarizations, by dopamine (30 μM) and baclofen (3 μM). *B*, 25 min after cell impalement with an electrode containing GTP-γ-S (1 mM) spontaneous firing had ceased and the membrane had hyperpolarized to −69 mV. Transient hyperpolarizations resulted from current pulses (50 pA, 2 s). Dopamine (30 μM) caused a further hyperpolarization to −74 mV and a fall in input resistance which did not reverse on wash-out. Baclofen (3 μM) hyperpolarized the membrane to −83 mV, accompanied by a further fall in input resistance, with recovery of membrane potential to only −80 mV on wash-out of drug. The effects of subsequent applications of dopamine (30 μM) and baclofen (30 μM) on membrane potential recovered on wash-out, but only to −80 mV.

spontaneous action potentials, although single spikes could be evoked by depolarizing current pulses. Twenty to thirty minutes after cell impalement, these cells had resting membrane potentials of $-59{\cdot}8 \pm 4{\cdot}0$ mV and input resistances of 105 ± 17 MΩ. Baclofen (3 μM) and dopamine (30 μM) (applied 15 min or more after impalement) were without effect on membrane potential or input resistance; GABA (3 mM) produced a large depolarization.

Within 10–15 min of impalement with electrodes containing 2 mM-GTP-γ-S, cells fired spontaneously. Baclofen (3 μM) produced a hyperpolarization of $9{\cdot}3 \pm 0{\cdot}7$ mV ($n = 4$) when applied within 15 min of impalement; this recovered only partially on

wash-out to a new resting membrane potential value of $-65{\cdot}3 \pm 4{\cdot}4$ mV. At this potential cells did not fire spontaneously and input resistance was $130 \pm 15{\cdot}3$ MΩ ($n = 3$). Subsequent application of either dopamine (30 μM) or baclofen (3 μM) either had no effect or caused small (2–3 mV) reversible hyperpolarizations.

Four cells impaled with electrodes containing GTP-γ-S (1 mM) initially fired spontaneously, but hyperpolarized to $-68 \pm 2{\cdot}0$ mV during the first 8–25 min of impalement; the input resistance was then $90 \pm 17{\cdot}3$ MΩ. Depolarization with constant direct current induced regular spontaneous firing. Dopamine (30 μM) or baclofen (3 μM) produced a further membrane hyperpolarization that did not reverse when application was discontinued (Fig. 7*B*). Subsequent applications of either dopamine or baclofen (15–31 min after impalement) hyperpolarized the membrane further; these hyperpolarizations only partially reversed, bringing the membrane potential to $-79 \pm 1{\cdot}2$ mV (input resistance was $73 \pm 16{\cdot}4$ MΩ; Fig. 7*B*). Further application of dopamine or baclofen produced hyperpolarizations which completely reversed on wash-out to this previous level of around −80 mV (Fig. 7*B*). This reversible hyperpolarization could be elicited repeatedly. Application of GABA (1 or 3 mM) and muscimol (10 μM) to neurones thus hyperpolarized caused depolarizations with pronounced falls in input resistance. In brief, cells impaled with microelectrodes containing 1 mM-GTP-γ-S exhibited partially irreversible hyperpolarizations in response to either dopamine or baclofen, accompanied by an increase in membrane conductance.

Effects of pertussis toxin pre-treatment

Recordings were made from nine cells in slices taken from the brains of four different rats injected intracerebroventricularly with pertussis toxin. Baclofen (3 μM) produced hyperpolarizations of $10{\cdot}5 \pm 1{\cdot}8$ mV (eight cells) which were not significantly different in amplitude compared to its effect ($12{\cdot}9 \pm 0{\cdot}6$ mV; sixty-one cells) on cells from untreated rats. Similarly, hyperpolarizations in response to dopamine (30 μM) of $4{\cdot}9 \pm 1{\cdot}4$ mV (seven cells) were not significantly different from the values of $6{\cdot}0 \pm 0{\cdot}45$ mV (thirty-eight cells) seen in cells from untreated rats. Innis & Aghajanian (1987) found that the inhibitory action of dopamine on rat substantia nigra zona compacta neurones was abolished by prior injection of pertussis toxin directly into the zona reticulata.

DISCUSSION

Baclofen increases membrane potassium conductance

The basis for confidence that baclofen increases a potassium conductance is that the membrane potential at which the outward membrane current or hyperpolarization produced by baclofen reverses in polarity is dependent upon the extracellular potassium concentration (Fig. 3*B*), the sensitivity of the baclofen response to barium and to high concentrations of TEA (Fig. 4). The potassium dependence of the action of baclofen in hippocampal pyramidal neurones is well established (Newberry & Nicoll, 1985; Gähwiler & Brown, 1985; Inoue, Matsuo & Ogata, 1985) and has also been reported in neurones of the dorsolateral septal nucleus (Stevens, Gallagher & Shinnick-Gallagher, 1985), frontal neocortex (Howe, Sutor &

Zieglgänsberger, 1987) and the nucleus locus coeruleus (Osmanović & Shefner, 1988). Abolition of the response to baclofen by barium (0·3–3 mM) has been reported previously by Newberry & Nicoll (1985) and the relative insensitivity to millimolar concentrations of TEA by Inoue *et al.* (1985) in hippocampal pyramidal cells.

The results of the experiments with solutions that block calcium currents indicate that calcium entry is not required for the action of baclofen, as also previously observed by Newberry & Nicoll (1985). This finding also suggests that release of transmitters from other cells is not necessary for the observed actions of baclofen. However, in general agreement with Blaxter, Carlen, Davies & Kujtan (1986), prolonged treatment with these solutions did produce significant reductions in baclofen hyperpolarizations. Due to the difficulty in reversing the effects of such treatments, both on the response to baclofen and on other properties of the cells, we are reluctant to conclude, contrary to Blaxter *et al.* (1986), that the potassium conductance is activated by calcium entry. Gähwiler & Brown (1985) and Andrade *et al.* (1986) also concluded that extracellular calcium was not required for the hyperpolarizing action of baclofen.

Baclofen acts on the $GABA_B$ receptor

In the absence of a specific $GABA_B$ antagonist, demonstration of the selectivity of baclofen for $GABA_B$ receptors was based upon the inability of the selective $GABA_A$ receptor agonist (muscimol) and antagonist (bicuculline) respectively either to mimic or reduce the effect of baclofen (Fig. 5) and upon the greater potency of the (−) over the (+) enantiomer of baclofen (Fig. 2). The action of baclofen could be mimicked by GABA in the presence of bicuculline, although bicuculline did not affect the action of baclofen. It is difficult, however, to be very precise in an assessment of the relative contribution to the overall action of GABA of the components resulting from $GABA_A$ (chloride conductance increase) and $GABA_B$ (potassium conductance increase) receptors; all that can be said is that both effects are clearly present on all the neurones.

Baclofen increases the same potassium conductance as dopamine

The main reason for considering the potassium conductance increased by baclofen to be the same as that coupled to D_2 receptors is the occlusion of the dopamine outward current by a maximal baclofen outward current in the same neurone (Fig. 6). Further similarities in the responses to dopamine and baclofen were sensitivities to TEA and barium (Fig. 4), independence from calcium entry (present study; Lacey *et al.* 1987) and the effects of intracellular GTP-γ-S. Another common phenomenon was the decline in response amplitude which was seen only when the response levels approached about 80% of the maximum. The method of occlusion of current induced by one agonist by the current induced by an agonist at a different receptor has previously been used to demonstrate that μ-opioid and α_2-adrenoceptors are coupled to the same potassium channel in locus coeruleus neurones (North & Williams, 1985) and that 5-hydroxytryptamine (probably 5-HT_1 type) and $GABA_B$ receptors share a potassium conductance in rat hippocampal pyramidal neurones (Andrade *et al.* 1986).

$GABA_B$ and D_2 receptor–channel coupling involves a G-protein

The evidence for the assertion that both $GABA_B$ and D_2 receptors are coupled to a potassium channel by a G-protein is the partial irreversibility of the baclofen and dopamine hyperpolarizations in cells impaled with electrodes containing 1 mM-GTP-γ-S (Fig. 7*B*). Successive applications of dopamine and baclofen irreversibly hyperpolarized the membrane to about −80 mV, but subsequent agonist application produced hyperpolarizations that reversed back to −80 mV on wash-out of the drug.

There are several difficulties associated with the interpretation of these experiments as follows. (1) The concentration of GTP-γ-S in the cytoplasm is unknown. (2) The cytoplasmic concentration of GTP-γ-S is likely to be non-uniform: higher in the soma than in more distal dendritic regions. (3) GTP-γ-S may permanently activate G-proteins in the absence of agonist. (4) Dopamine and GABA released from other neurones in the slice may cause the hyperpolarization observed prior to adding exogenous agonists. (5) A number of other intracellular processes might be expected to be sensitive to GTP-γ-S. The higher concentration used (20 mM) blocked spontaneous action potentials; the lower concentrations sometimes caused a regular burst firing of a kind not observed with electrodes containing only KCl. Despite these difficulties, the results point to the need for GTP hydrolysis in termination of the action of dopamine and baclofen. These findings using GTP-γ-S-containing electrodes and their effects on a receptor-activated potassium conductance increase are essentially similar to those reported in studies of rat hippocampal CA1 pyramidal cells with respect to 5-HT_1 and $GABA_B$ receptors (Andrade *et al.* 1986), rat locus coeruleus neurone μ-opioid receptors (North, Williams, Surprenant & Christie, 1987), guinea-pig submucous plexus neurone δ-opioid receptors (North *et al.* 1987), somatostatin receptors (Mihara, North & Surprenant, 1987) and α_2-adrenoceptors (Surprenant & North, 1988), and *Aplysia* abdominal ganglion cell dopamine, histamine and acetylcholine receptors (Sasaki & Sato, 1987).

The lack of effect of intracerebroventricular injection of pertussis toxin on the ability of both baclofen and dopamine to induce hyperpolarizations would appear to indicate that the G-protein involved is not sensitive to the toxin. Intracerebroventricular injection of pertussis toxin prevents the effects of both α_2-adrenoceptor and μ-opioid receptor agonists in rat locus coeruleus neurones (Aghajanian & Wang, 1986) and of 5-HT_1 and $GABA_B$ receptor agonists in rat hippocampal CA1 pyramidal cells (Andrade *et al.* 1986), all of which are due to a potassium conductance increase. However, the nucleus locus coeruleus and the hippocampal CA1 pyramidal cell layer are both periventricular structures; the substantia nigra is not and may have been unaffected by pertussis toxin due to limits on its diffusion from the ventricles. This may account for the difference between the present findings and those of Innis & Aghajanian (1987).

We wish to thank Dr W. F. Colmers for treating rats with pertussis toxin and Dr J. T. Williams for helpful advice and discussion. The work was supported by a grant from the Alcohol, Drug Abuse and Mental Health Administration of the United States Department of Health and Human Services MH40416.

REFERENCES

AGHAJANIAN, G. K. & WANG, Y. Y. (1986). Pertussis toxin blocks the outward currents evoked by opiate and α_2-agonists in locus coeruleus neurons. *Brain Research* **371**, 390–394.

ANDRADE, R., MALENKA, R. C. & NICOLL, R. A. (1986). A G protein couples serotonin and $GABA_B$ receptors to the same channels in hippocampus. *Science* **234**, 1261–1265.

AXELROD, J. (1971). Noradrenaline: fate and control of its biosynthesis. *Science* **173**, 598–606.

BLAXTER, T. J., CARLEN, P. L., DAVIES, M. F. & KUJTAN, P. W. (1986). γ-Aminobutyric acid hyperpolarizes rat hippocampal pyramidal cells through a calcium-dependent potassium conductance. *Journal of Physiology* **373**, 181–194.

BOWERY, N. G., HILL, D. R. & HUDSON, A. L. (1983). Characteristics of $GABA_B$ receptor binding sites on rat whole brain synaptic membranes. *British Journal of Pharmacology* **78**, 191–206.

BOWERY, N. G., HILL, D. R., HUDSON, A. L., DOBLE, A., MIDDLEMISS, D. N., SHAW, J. & TURNBULL, M. (1980). (−)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature* **283**, 92–94.

BROWN, D. A., COLLINS, G. G. S. & GALVAN, M. (1980). Influence of cellular transport on the interaction of amino acids with gamma-aminobutyric acid (GABA)-receptors in the isolated olfactory cortex of the guinea-pig. *British Journal of Pharmacology* **68**, 251–262.

CARLSSON, A. & LUNDQVIST, M. (1963). Effect of chlorpromazine and haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta pharmacologica toxicologica* **20**, 140–144.

DRAY, A. (1979). The striatum and substantia nigra: a commentary on their relationships. *Neuroscience* **4**, 1407–1439.

FONNUM, F. & STORM-MATHISEN, J. (1978). Location of GABA-ergic neurons in the CNS. In *Handbook of Psychopharmacology*, vol. 9, *Chemical Pathways in the Brain*, ed. IVERSEN, L. L., IVERSEN, S. D. & SNYDER, S. H., pp. 357–401. New York: Plenum Press.

GÄHWILER, B. H. & BROWN, D. A. (1985). $GABA_B$-receptor-activated K^+ current in voltage-clamped CA_3 pyramidal cells in hippocampal cultures. *Proceedings of the National Academy of Sciences of the U.S.A.* **82**, 1558–1562.

HILL, D. R. & BOWERY, N. G. (1981). ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline-insensitive $GABA_B$ sites in rat brain. *Nature* **290**, 149–152.

HOWE, J. R., SUTOR, B. & ZIEGLGÄNSBERGER, W. (1987). Baclofen reduces post-synaptic potentials of rat cortical neurones by an action other than its hyperpolarizing action. *Journal of Physiology* **384**, 539–569.

INNIS, R. B. & AGHAJANIAN, G. K. (1987). Pertussis toxin blocks autoreceptor-mediated inhibition of dopaminergic neurons in rat substantia nigra. *Brain Research* **411**, 139–143.

INOUE, M., MATSUO, T. & OGATA, N. (1985). Baclofen activates voltage-sensitive and 4-amino-pyridine sensitive K^+ conductance in guinea-pig hippocampal pyramidal cells maintained *in vitro*. *British Journal of Pharmacology* **84**, 833–841.

LACEY, M. G., MERCURI, N. B. & NORTH, R. A. (1987). Dopamine acts on D_2 receptors to increase potassium conductance in neurones of the rat substantia nigra compacta. *Journal of Physiology* **392**, 397–416.

MIHARA, NORTH & SURPRENANT (1987). Somatostatin increases an inwardly rectifying potassium conductance in guinea-pig submucous plexus neurones. *Journal of Physiology* **390**, 335–355.

NEWBERRY, N. R. & NICOLL, R. A. (1985). Comparison of the action of baclofen with γ-amino-butyric acid on rat hippocampal pyramidal cells *in vitro*. *Journal of Physiology* **360**, 161–185.

NORTH, R. A. & WILLIAMS, J. T. (1985). On the potassium conductance increased by opioids in rat locus coeruleus neurones. *Journal of Physiology* **364**, 265–280.

NORTH, R. A., WILLIAMS, J. T., SURPRENANT, A. & CHRISTIE, M. J. (1987). μ and δ receptors belong to a family of receptors that are coupled to potassium channels. *Proceedings of the National Academy of Sciences of the U.S.A.* **84**, 5487–5491.

OLPE, H.-R., KOELLE, W. P., WOLF, P. & HAAS, H. L. (1977). The action of baclofen on neurons of the substantia nigra and of the ventral tegmental area. *Brain Research* **134**, 577–580.

OSMANOVIĆ, S. S. & SHEFNER, S. A. (1988). Baclofen increases the potassium conductance of rat locus coeruleus neurons recorded in brain slices. *Brain Research* **438**, 124–136.

PINNOCK, R. D. (1984). Hyperpolarizing action of baclofen on neurones in the rat substantia nigra slice. *Brain Research* **322**, 337–340.

SASAKI, K. & SATO, M. (1987). A single GTP-binding protein regulates K^+-channels coupled with dopamine, histamine and acetylcholine receptors. *Nature* **325**, 259–262.

STEVENS, D. R., GALLAGHER, J. P. & SHINNICK-GALLAGHER, P. (1985). Further studies on the action of baclofen on neurons of the dorsolateral septal nucleus of the rat, *in vitro*. *Brain Research* **385**, 360–363.

SURPRENANT, A. & NORTH, R. A. (1988). Mechanism of synaptic inhibition by noradrenaline acting at α_2 adrenoceptors. *Proceedings of the Royal Society* B (in the Press).

VAN DEN POL, A. N., SMITH, A. D. & POWELL, J. F. (1985). GABA axons in synaptic contact with dopamine neurones in the substantia nigra: double immunocytochemistry with biotin-peroxidase and protein A-colloidal gold. *Brain Research* **384**, 146–154.