Modulation of plateau properties in dorsal horn neurones in a slice preparation of the turtle spinal cord

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1. Modulation of plateau properties in dorsal horn neurones was studied in a transverse slice preparation of the spinal cord of the turtle. In plateau-generating neurones high frequency stimulation of the ipsilateral dorsal root (10–20 Hz, 0·5–2 min) produced a slow depolarization (2·9 ± 0·6 mV, mean ± S.E.M.; $n = 6$) and enhanced the properties mediated by dihydropyridine-sensitive Ca^{2+} channels. The tetanic stimulus facilitated wind-up and after-discharges even when fast synaptic transmission was blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10–20 μM), (±)-2-amino-5-phosphonopentanoic acid (AP5, 100 μM), bicuculline (10–20 μM) and strychnine (5–20 μM).

2. Application of cis-(±)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD, 10–50 μM) produced a slow depolarization (5·9 ± 0·5 mV, $n = 21$) accompanied by an increase in input resistance (28·8 ± 5·1 %, $n = 12$).

3. ACPD increased the excitability by facilitating the plateau properties. In the presence of tetrodotoxin (TTX, 1 μM) a lower threshold and a slower decay of the plateau potential were observed. These effects resulted in facilitation of wind-up and prolonged after-discharges.

4. All ACPD-induced effects were blocked by α-methyl-4-carboxyphenylglycine (MCPG, 0·5–1 mM), a selective antagonist of metabotropic glutamate receptors. The selective agonist for the type I metabotropic glutamate receptor ((*RS*)-3,5-dihydrophenylglycine (DHPG, 50 μM)) reproduced all the effects of ACPD.

5. Application of a supposed neuromodulator, substance P (1–2 μM) produced a transient depolarization (4 ± 0·6 mV) lasting 4–6 min during continued application of substance P. Variable effects on the input resistance were observed, a slight increase (12 ± 2 %) being the most frequent. In 61 % of the cells, substance P induced a clear increase in excitability with no detectable change in input resistance or membrane potential.

6. The effects of substance P on plateau properties were indistinguishable from those produced by ACPD. Unlike the transient depolarization, the facilitation of the plateau properties persisted in the presence of the agonist.

7. The substance P-induced facilitation of the plateau potential was blocked by GR 82334 (5–10 μM), a selective NK-1 tachykinin-receptor antagonist, and was not affected by MEN 10376 (2 μM), a selective NK-2 antagonist.

8. The facilitation of plateau properties produced by dorsal root stimulation was also reduced by antagonists of metabotropic glutamate receptors and NK-1 tachykinin receptors.

9. We propose that modulation of postsynaptic plateau properties in dorsal horn neurones by activation of type I metabotropic glutamate receptors and NK-1 tachykinin receptors is involved in processing nociceptive information.

Synaptic integration of somato-sensory information in the dorsal horn of the spinal cord involves complex interactions between primary afferents, intrinsic neurones and descending pathways (Willis & Coggeshall, 1991). These interactions exhibit a remarkable degree of plasticity as revealed by phenomena like habituation (Egger, 1978), dynamic receptive fields (Cook, Wolf, Wall & McMahon, 1987) and central sensitization to pain (Woolf, 1983). Neither sites nor

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mechanisms of plasticity are well known but both activity-dependent changes (Dubner & Ruda, 1992) and modulation by transmitters contribute (Zieglgänsberger & Tölle, 1993) to these processes.

The intrinsic properties of neurones are major determinants of function in neural networks (Llinás, 1988) and form a potential site for plasticity. In the turtle, the active response properties of a subset of dorsal horn neurones are dominated by a plateau potential mediated by a dihydropyridine-sensitive, non-inactivating Ca^{2+} conductance (Russo & Hounsgaard, 1996). This plateau potential activates with slow kinetics near the resting membrane potential and plays an important part in synaptic integration by shaping primary afferent inputs in a time- and voltage-dependent manner (Russo & Hounsgaard, 1996). Indeed, the increasing activation of the Ca^{2+} plateau potential is the main cellular mechanism involved in wind-up of the response to repetitive stimulation of primary afferents (Russo & Hounsgaard, 1994), a type of short-term plasticity thought to be an intermediate step in the development of central sensitization to pain (McMahon, Lewin & Wall, 1993).

The intrinsic properties of nerve cells are far from being rigid neuronal attributes but are finely tuned by transmitters and hormones (Kazmareck & Levitan, 1987). In the dorsal horn of the spinal cord several transmitter candidates are present both in primary afferents, elements intrinsic to the dorsal horn and fibres from supraspinal levels (Willis & Coggeshall, 1991). Many of these transmitters are mono-amines or peptides and are believed to be important in sensory processing by mediating slow synaptic potentials or by modulating synaptic efficacy (Urban, Thompson & Dray, 1994). Although neuromodulators can also affect voltage-operated channels in dorsal horn cells (Murase, Ryu & Randic, 1989), the significance for the intrinsic response properties is unknown. In the present experiments we have investigated the modulation of plateau-generating neurones by transmitters released by primary afferents. We find that the intrinsic properties of these cells are modulated by primary afferent activity in ways that enhance wind-up and after-discharges. These effects were mimicked by activation of metabotropic glutamate receptors and NK-1 tachykinin receptors and strongly reduced by their selective receptor antagonists.

Some of the results have been published in abstract form (Russo, Nagy & Hounsgaard, 1994) and more recently confirmed in the rat (Morisset & Nagy, 1996).

METHODS

Preparation

The preparation used in this study has been described elsewhere (Russo & Hounsgaard, 1996). Briefly, adult turtles (*Pseudemys scripta*, 15–20 cm carapace length) were anaesthetized with sodium pentobarbitone (100 mg kg^{-1}, I.P.). After complete unresponsiveness to painful stimuli was achieved, a window in the plastron was opened with an oscillating saw (Aesculap, Tütlingen, Germany) and the blood removed by intraventricular perfusion with Ringer solution (6–10 °C). The lumbar enlargement was dissected and thick (1·5–2 mm) transverse slices with dorsal roots attached were cut with a home-made slicing machine. After dissection the animal was killed by decapitation. For recording, the slice was glued to a piece of filter paper with cyanoacrylate and fixed to the bottom of the recording chamber (1 ml volume) with pieces of silver wire. The preparation was kept at room temperature (20–22 °C) and continuously superfused at a rate of 1 ml min^{-1} with Ringer solution having the following composition (mM): 120 NaCl, 5 KCl, 15 $NaHCO_3$, 3 $CaCl_2$, 2 $MgCl_2$, and 20 glucose. This solution was saturated with a gas mixture containing 2% CO_2–98% O_2 to obtain a pH of 7·6.

Electrophysiological recordings and stimulation

Conventional intracellular recordings in the current-clamp mode were performed with an amplifier equipped with an active bridge circuit and capacitance compensation (Axoclamp 2A). The electrodes for recording were pulled in a Flaming/Brown P-87 puller (Sutter Instruments) from borosilicate thin-walled glass (1·5 mm, o.d; 1·17 mm, i.d.; Clark Electromedical Instruments) and filled with 1 M potassium acetate and 1% biocytin (50–80 MΩ). The electrodes were positioned under visual guidance using a dissecting microscope (Leica Wild M3Z). In order to characterize the passive and active properties of neurones, rectangular pulses of current were injected through the electrode by driving the amplifier with a programmable stimulator (Master-8, A.M.P.I., Jerusalem, Israel). The bridge was balanced by cancelling the fast voltage jump at the onset and offset of the current pulse and bridge balance was continuously monitored throughout the experiment. Extracellular stimulation was performed by applying brief (0·5 ms) constant current pulses (Iso-Flex, A.M.P.I.) to the ipsilateral dorsal root by means of a suction electrode.

Database and processing

The criteria for selection of cells were an action potential of more than 60 mV (measured from peak to peak) and the ability to sustain increasing firing frequency of action potentials in response to long-lasting depolarizing current pulses, indicating the presence of a plateau potential (Russo & Hounsgaard, 1996). A few cells without this firing pattern were studied for comparison.

During experiments the recordings were monitored on a digital oscilloscope (Gould 420) and simultaneously stored on tape (Racal, 0–5 kHz bandwidth) for off-line analysis. The recorded data were digitized at 800 kHz using the envelope function of a digital oscilloscope (Hioki 8851) and subsequently transferred to a personal computer. Numerical values are expressed as means ± the standard error of the mean (S.E.M.).

Drugs

In some experiments the following drugs were added to the normal medium: tetrodotoxin (TTX, 1 μM; Sigma); 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10–20 μM; RBI); strychnine (5–20 μM; Sigma); bicuculline (10–50 μM; Sigma); (±)-2-amino-5-phosphonopentanoic acid (AP5, 100 μM; RBI); substance P (1–2 μM; RBI); cis-(±)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD, 10–50 μM; RBI); (*RS*)-3,5-dihydrophenylglycine (DHPG, 50 μM; Tocris Cookson); α-methyl-4-carboxyphenylglycine (MCPG, 0·5–1 mM; RBI); GR 82334 (5–10 μM, Tocris Cookson); and MEN 10376 (2 μM; RBI).

RESULTS

Modulation of plateau-generating neurones by primary afferent stimulation

Prolonged high frequency stimulation of the dorsal root (DR) (0·5–2 min at 10–20 Hz) changed the intrinsic response properties of plateau-generating neurones in the ipsilateral dorsal horn. The results obtained in sixteen cells with robust synaptic responses to DR stimulation are illustrated in Figs 1 and 2. In order to avoid a general increase in activity which would blur the effects on the postsynaptic cell, fast synaptic transmission was blocked by CNQX (10 μM), AP5 (100 μM), strychnine (5 μM) and bicuculline (20 μM) added to the normal medium. In this transmitter–antagonist medium the fast synaptic response to a primary afferent stimulus was blocked completely in three cells while a CNQX-resistant component was left in the remaining thirteen cells, as exemplified in Fig. 1*A a* and *b*. To avoid activity-dependent changes in the postsynaptic membrane properties, the cell was hyperpolarized by 20–30 mV from rest during the high frequency stimulation. The cell shown in Fig. 1*A–C* did not fire spontaneously before the tetanic DR stimulation (Fig. 1*B*) and responded tonically to a depolarizing current

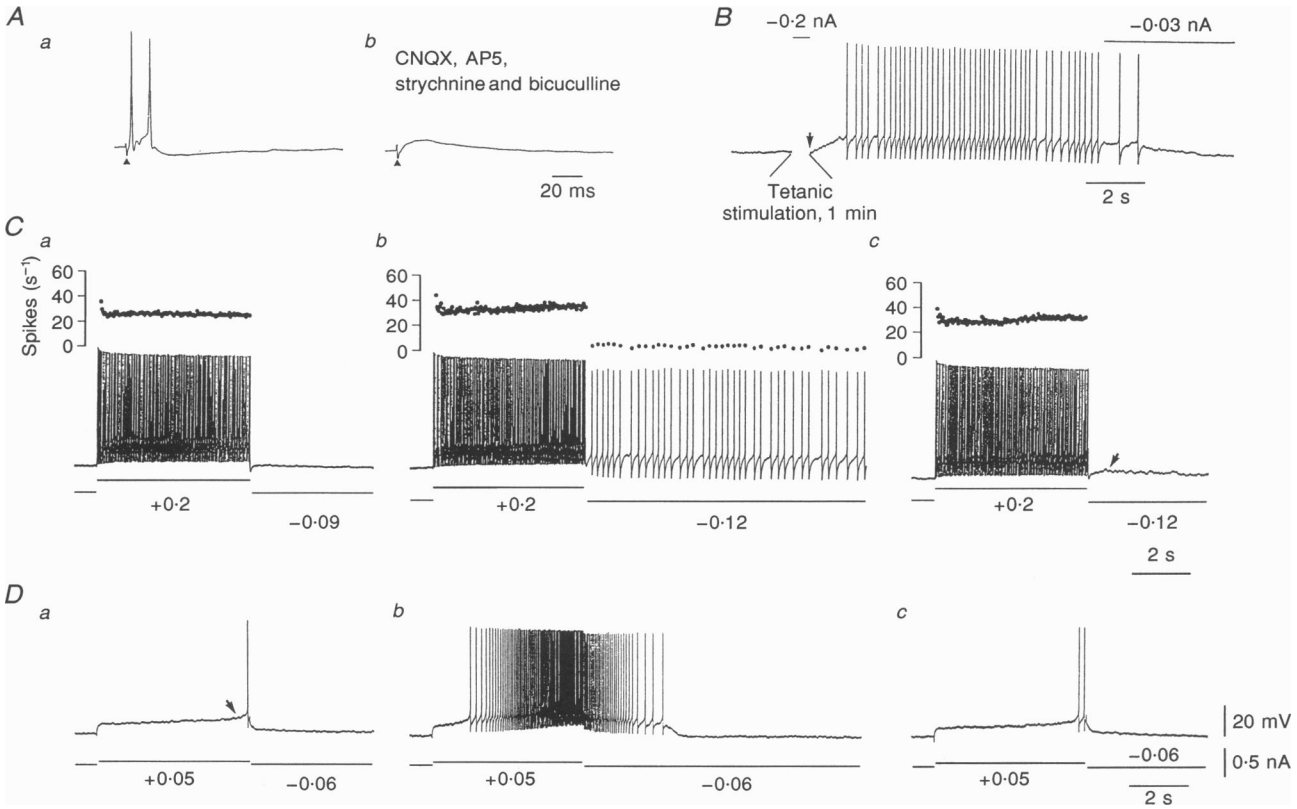

Figure 1. Modulation of plateau properties by dorsal root (DR) stimulation

A, response to a DR stimulation (40 μA, ▲) before (*a*) and after (*b*) addition of CNQX (10 μM), AP5 (100 μM), strychnine (5 μM) and bicuculline (20 μM). *B*, in the presence of the transmitter antagonists, the DR was stimulated at 10 Hz for 1 min. During the stimulation the cell was hyperpolarized with negative current (−0·2 nA). Upon release of the bias current after DR stimulation (arrow), the cell slowly depolarized and fired action potentials spontaneously. Slight application of hyperpolarizing holding current (−0·03 nA, long horizontal bar) suppressed the spontaneous discharge. *C*, the plateau properties were potentiated by primary afferent activity. Before DR stimulation the cell responded to a depolarizing current pulse with a tonic discharge (*a*). Instantaneous frequency plots (IFP) are shown on top of recordings. The same stimulus applied immediately after the tetanic stimulation produced a stronger response during the pulse and a prolonged after-discharge (*b*). Ten minutes after the DR stimulation a partial recovery of the response was observed (*c*) but the cell still showed an increased acceleration during the depolarizing pulse and a subthreshold after-potential (arrow). All the stimuli were applied at the same membrane potential. *D*, decreased threshold for plateau potential activation after DR stimulation. The intensity of a depolarizing current pulse was adjusted to produce a weak activation of the plateau potential at the end of the pulse (*a*, arrow). After DR stimulation (100 μA at 20 Hz, 1 min) the same initial depolarization produced a powerful activation of the plateau potential (*b*). Normal excitability recovered after 17 min of rest (*c*). Fast synaptic transmission blocked in *B*, *C* and *D*. *D* is taken from a different cell.

pulse of moderate amplitude (Fig. 1*Ca*). More intense depolarization produced an initial phase of accelerating firing frequency, showing that the cell was able to generate plateau potentials (not illustrated). When the hyperpolarizing holding current was released after tetanic DR stimulation for 1 min (Fig. 1*B*, arrow), the cell slowly depolarized (8/16 cells; 2·9 ± 0·6 mV, $n = 6$) and initiated spontaneous firing (2/8 cells). After adjusting the holding current to re-establish the pre-stimulus level of membrane potential, the same depolarizing current pulse as in Fig. 1*Ca* evoked a stronger response (13/16 cells) with an increased acceleration of firing and a prolonged after-discharge (Fig. 1*Cb*). Ten minutes after the DR stimulation, the promoted after-discharge had disappeared but the response was still potentiated compared with control, as revealed by the higher frequency during the stimulus and the larger after-potential (Fig. 1*Cc*, arrow). In six out of thirteen cells, the DR stimulation did not produce a slow depolarization but still potentiated the plateau properties of dorsal horn neurones.

The data shown in Fig. 1*C* suggested that after prolonged activation of primary afferents the plateau potential activated at a less depolarized membrane potential. This is illustrated more clearly by the records in Fig. 1*D* from another cell. In control conditions the strength of the depolarizing current pulse was adjusted to activate a plateau potential at the end of the pulse (Fig. 1*Da*, arrow). After tetanic DR stimulation (20 Hz, 1 min) the plateau potential activated after a short delay and produced a strong burst of action potentials (APs) by the end of the pulse (Fig. 1*Db*). Note, however, that the depolarization attained at the onset of the pulse was essentially the same as the control. Also, an after-discharge appeared after the tetanus, which was not readily elicited in control conditions (Fig. 1*D a* and *b*). Seventeen minutes after DR stimulation the response had returned to control levels (Fig. 1*Dc*).

The modulation of plateau properties by primary afferent stimulation affected temporal integration in the dorsal horn neurones. The cell shown in Fig. 2 exhibited wind-up of the

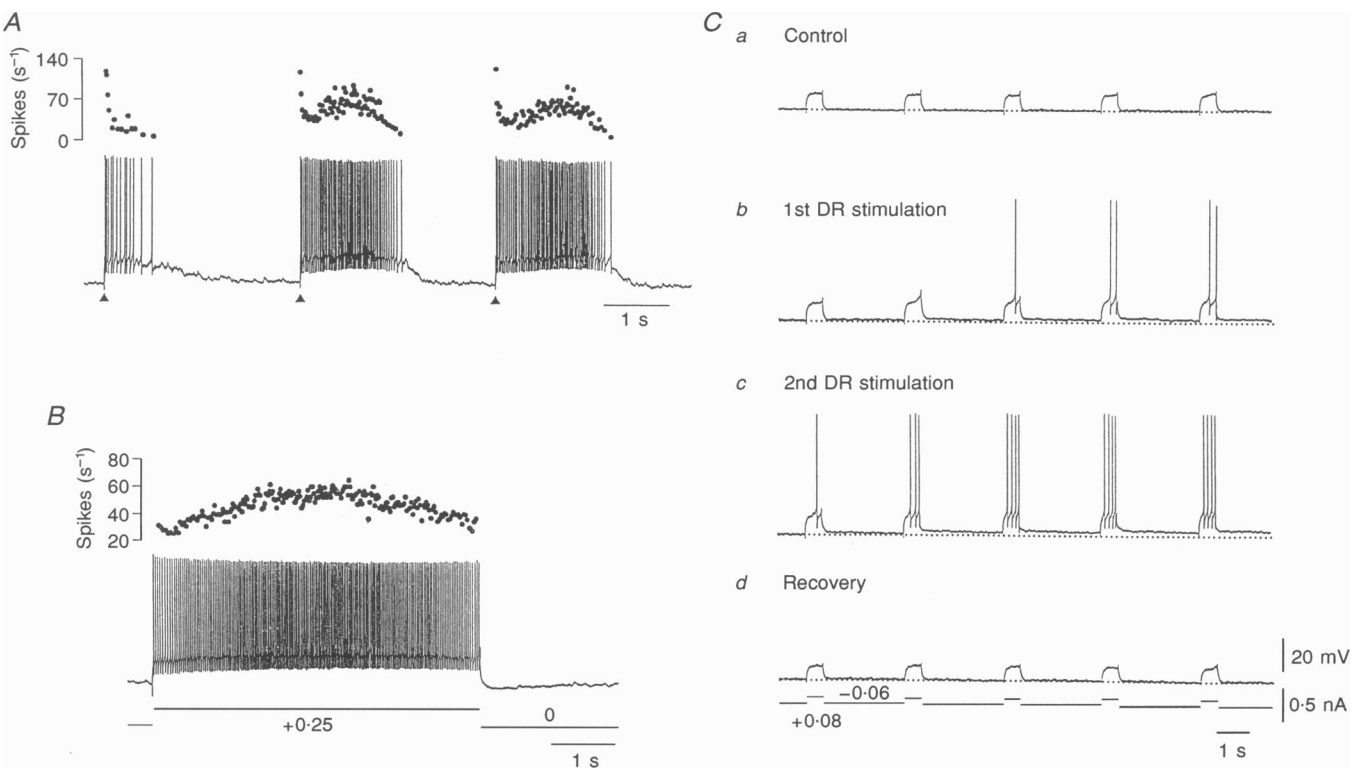

Figure 2. Facilitation of wind-up by DR stimulation

A, wind-up of the response to repetitive stimulation of the ipsilateral DR (▲) at 0·3 Hz. Notice the slow increase in firing frequency following the initial adaptation and the prolongation of the response to the 2nd and 3rd stimuli. *B*, the response to a depolarizing current pulse showed an incrementing firing frequency. *C*, prolonged high frequency stimulation of the DR facilitated intracellularly induced wind-up. With fast synaptic transmission blocked by CNQX (10 μM), AP5 (100 μM), bicuculline (20 μM) and strychnine (5 μM) a subthreshold protocol to produce wind-up (*a*) elicited a clear wind-up of the response after a single tetanic stimulation (20 Hz, 1 min) of the DR (*b*). A 2nd tetanus further increased wind-up (*c*). The normal excitability recovered after 15 min of rest (*d*). All data are taken from the same cell. In this and subsequent figures, dotted lines indicate the pre-stimulus membrane potential.

response to repetitive stimulation of the DR at 0·3 Hz (Fig. 2*A*). Note that the initial high frequency burst of APs (identical for the three stimuli) was followed by a long lasting response that increased in duration and firing frequency with each stimulus (Fig. 2*A*). The same profile of activity could be elicited with a depolarizing current pulse (Fig. 2*B*), indicating that activation of a plateau potential was responsible for the slow increase in firing rate observed during the wind-up of the response. After blockade of fast synaptic transmission, we applied repetitive depolarizing current pulses (500 ms, 0·3 Hz) with an intensity just subthreshold for eliciting intracellularly induced wind-up (Fig. 2*Ca*). The same stimulation protocol applied at the same membrane potential but immediately after DR stimulation (20 Hz, 1 min) produced a moderate wind-up of the response (Fig. 2*Cb*). A second tetanic DR stimulation further enhanced wind-up and cumulative depolarization in response to the repetitive depolarizing current pulses (Fig. 2*Cc*). The excitability returned to normal 15 min after the last DR tetanus (Fig. 2*Cd*).

Modulation of plateau properties by primary afferent transmitter candidates

Glutamate mediates fast synaptic transmission from primary afferents (Jessell & Dodd, 1989) but can also induce slow actions via metabotropic receptors (mGluRs) (Pin & Duvoisin, 1995). In addition, substance P is present in small diameter primary afferents and is thought to be a major neuromodulator in the dorsal horn (Otsuka & Yoshioka, 1993). We explored the possible involvement of these transmitter candidates in the modulation induced by stimulation of primary afferents by bath application of ACPD (a selective mGluR agonist) and substance P.

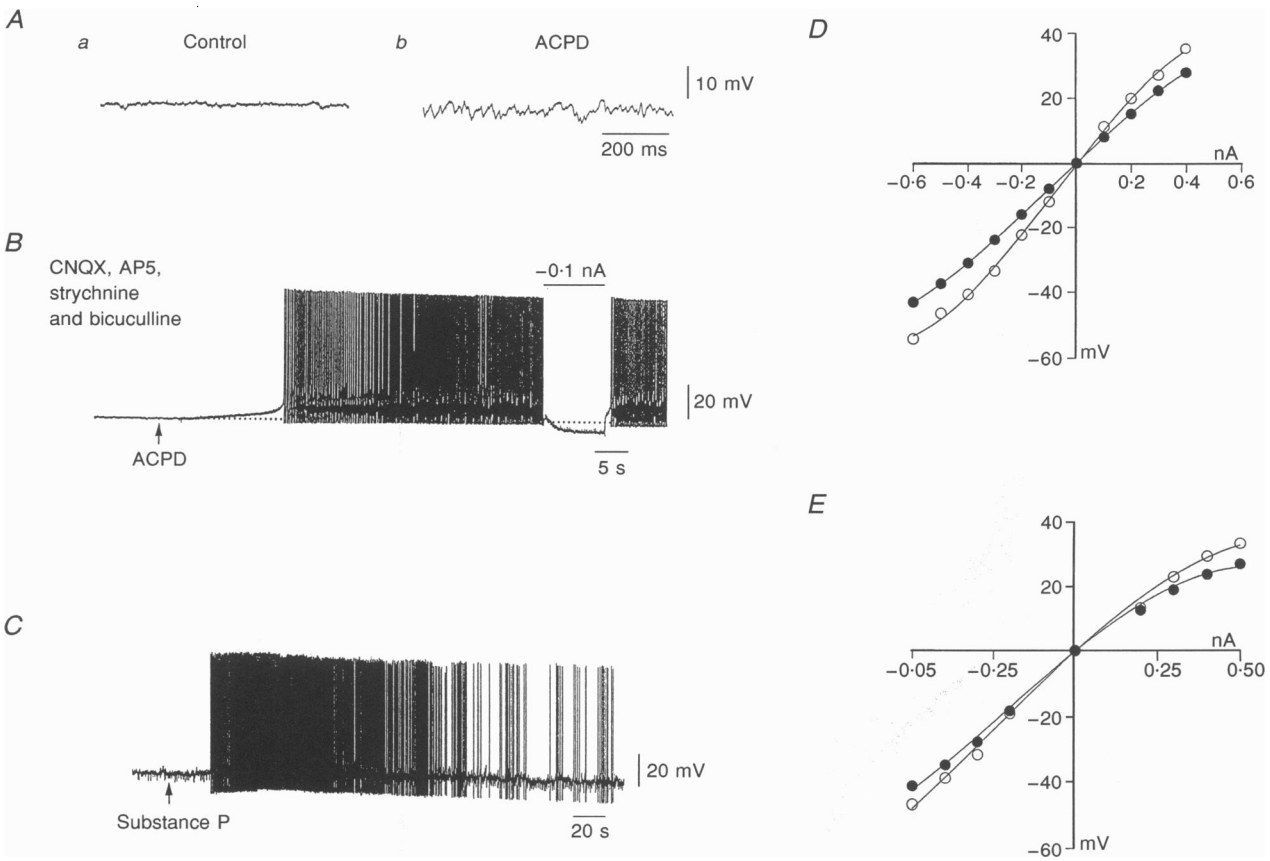

Figure 3. ACPD and substance P depolarized plateau-generating neurones

A, addition of ACPD (50 μM) to normal medium produced a marked increase in synaptic noise (*a* and *b*). *B*, under blockade of fast synaptic transmission with CNQX (10 μM), AP5 (100 μM), bicuculline (20 μM) and strychnine (10 μM) addition of ACPD (50 μM, arrow) elicited a slow depolarization leading to spontaneous firing. Horizontal bar on top of recording indicates injection of hyperpolarizing current. *C*, substance P (2 μM) added to normal medium (arrow) produced a depolarization and spontaneous firing of action potentials. Notice however, that the depolarization subsided slowly despite the presence of the peptide. *D*, current–voltage relationship obtained in the presence of TTX (1 μM). ACPD (50 μM) increased the input resistance (from 84 to 124 MΩ) but did not change the shape of the plot: Control, ●; ACPD, ○. *E*, current–voltage relationship obtained in the presence of TTX (1 μM; ●) showing a slight increase in the slope produced by substance P, ○. The lines represent 3rd-order regressions through data points. Data taken from different cells.

Table 1. Modulatory effects of ACPD and substance P

	Depolarization	Input resistance			Increase in excitability	No effect
		Increase	Decrease	No change		
ACPD						
Number of cells affected	100 % (21)	12/19	—	7/19	27/27	—
Mean effect	5·9 ± 0·54 mV	28·8 ± 5·1 %	—	—	—	—
Substance P						
Number of cells affected	72 % (18)	7/15	2/15	6/15	18/18	6/24
Mean effect	4 ± 0·6 mV	12 ± 2 %	10 and 25 %	—	—	—

Numbers in parentheses indicate n, the number of cells. Values expressed as means ± S.E.M.

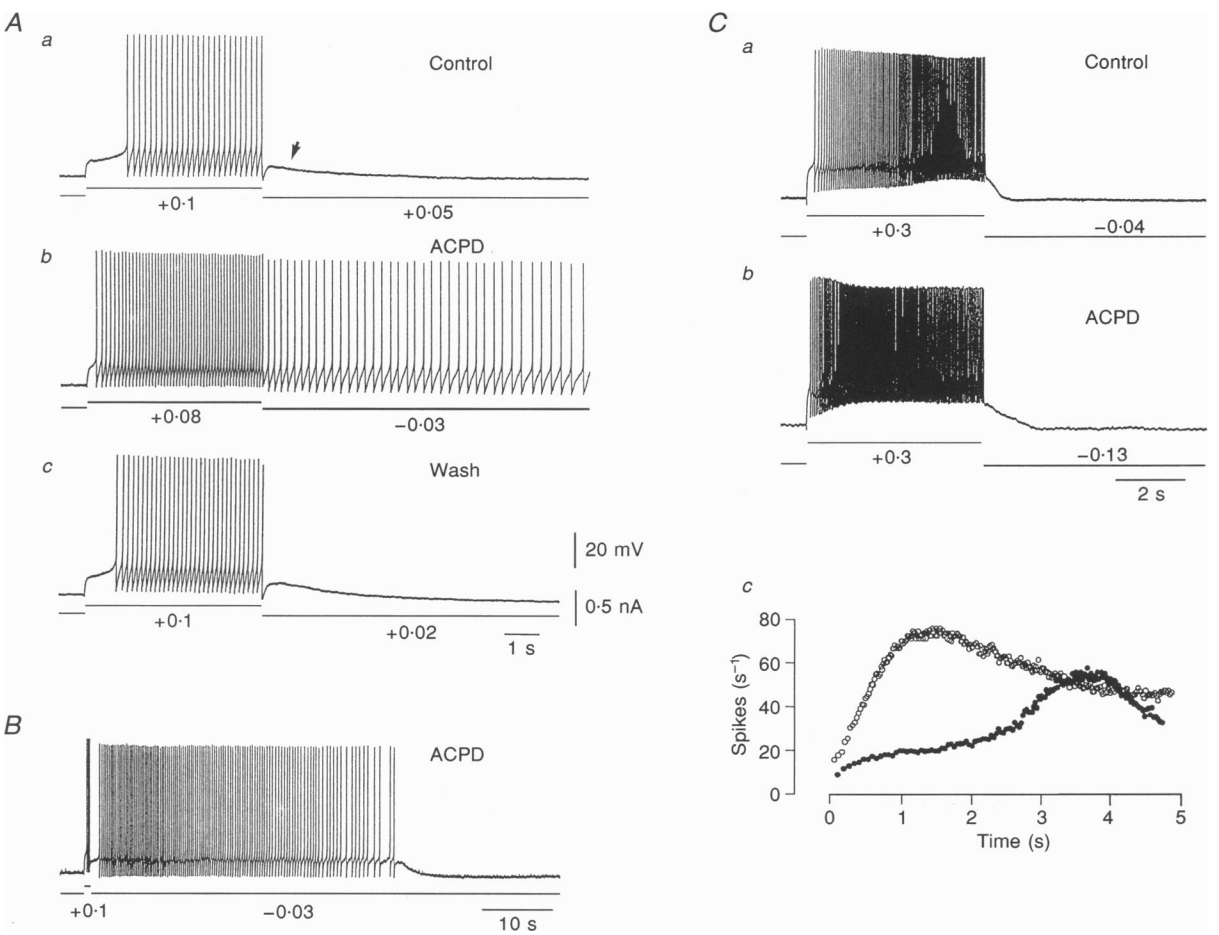

Figure 4. Modulation of plateau properties by ACPD

A, a depolarizing current pulse produced a delayed firing followed by a small depolarizing after-potential (arrow) due to plateau potential activation (*a*). After addition of ACPD (50 μM) a stronger response with a shorter delay was observed during the stimulus and was followed by a sustained after-discharge (*b*). The excitability recovered and the ability to display prolonged after-discharges disappeared after washing out ACPD (*c*). The injected currents were adjusted in order to apply the stimuli at the same membrane potential and to produce the same depolarization at the onset of the pulse. *B*, prolonged after-discharge induced by a short depolarizing current pulse in the presence of ACPD (50 μM). *C*, response to a depolarizing pulse before (*a*) and after (*b*) addition of ACPD (10 μM) in a cell with a strong plateau potential. The IFPs of the responses shown in (*a*) and (*b*) give an indication of the time course of the plateau potential (*c*: Control, ●; ACPD, ○). *A* and *B* are from the same cell. Fast synaptic transmission was blocked in all cases.

Addition of ACPD (10–50 μM) to the normal medium produced a marked increase in synaptic noise (Fig. 3*A*, $n = 6$). To avoid the increased synaptic activity we isolated the postsynaptic cell pharmacologically with either the transmitter antagonist medium ($n = 9$) or TTX (1 μM, $n = 11$). When fast synaptic transmission was blocked, ACPD produced a slow depolarization in all the cells recorded (Table 1). The depolarization ranged from 2·4 to 10 mV (mean, 5·9 ± 0·5 mV; $n = 21$) and was often sufficient to elicit spontaneous firing (Fig. 3*B*). As shown in Fig. 3, activation of the plateau potential by the ACPD-induced depolarization could also contribute to the sustained firing as suggested by the delayed re-activation of firing after a hyperpolarizing current pulse (Fig. 3*B*, horizontal bar at the top). In twelve out of nineteen cells, the depolarization induced by ACPD was associated with an increase in input resistance ranging from 4·7 to 52% (28·8 ± 5·1, $n = 12$). In the cell shown in Fig. 3*D* the input resistance increased from 84 to 124 MΩ. The change in input resistance was independent of the membrane potential over a wide range of potentials around rest (Fig. 3*D*).

Substance P (1–2 μM) produced qualitatively the same effects as ACPD. A small depolarization was observed in 72% of cells (13/18 cells, Table 1), ranging from 2 to 7·2 mV (Fig. 3*C*). However, in all cells, the depolarization subsided after 3–5 min in the presence of substance P, whereas the neurones remained depolarized as long as ACPD was present. The effect of substance P on the input resistance was variable. The most frequent effect (47% of cells, Table 1) was a small increase in input resistance (12 ± 2%) evidenced by an increase in the slope of the current–voltage relationship (from 93 to 106 MΩ in Fig. 3*E*). No change in input resistance was observed in 40% of neurones (6/15 cells), while a decrease was observed in two neurones (from 64 to 48 MΩ and 52 to 47 MΩ). Substance P had no effect in six cells (Table 1).

Effects of ACPD on the excitability of plateau-generating neurones

In addition to the slow depolarization and increase in input resistance, ACPD increased the excitability of plateau-generating neurones and promoted the plateau potential in a similar manner to that observed on tetanic stimulation of primary afferents. Figure 4*A* shows a cell in which a prolonged depolarizing current pulse produced the typical behaviour of a plateau neurone, with delayed firing and slow depolarizing after-potential subthreshold for AP generation (Fig. 4*A a*). Addition of ACPD (50 μM) reduced the delay and increased the frequency of firing elicited during the pulse which was followed by a prolonged after-discharge (Fig. 4*A b*). Notice that to avoid any change in excitability

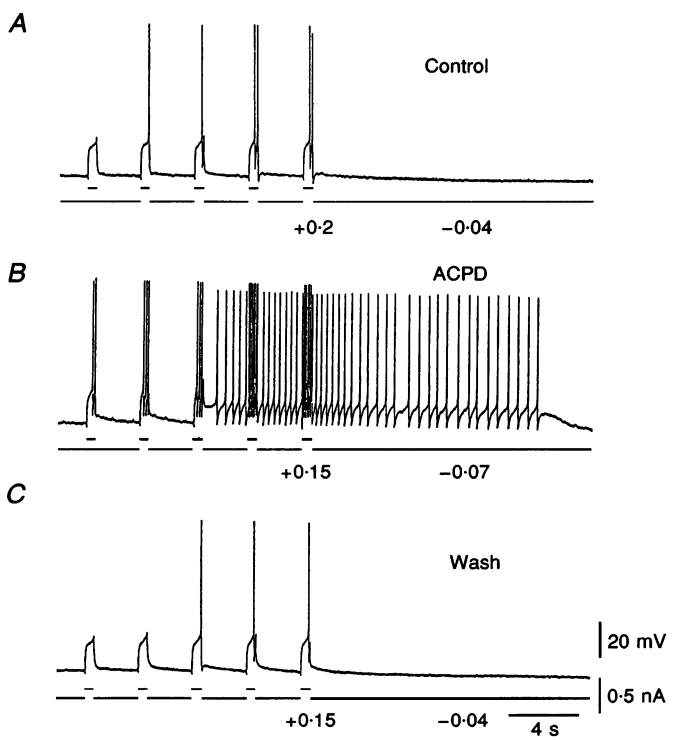

Figure 5. Activation of metabotropic glutamate receptor potentiated wind-up generation

A, a sequence of depolarizing current pulses produced a mild wind-up of the response in control conditions. *B*, ACPD (50 μM) greatly increased the wind-up of the response, producing a prolonged after-discharge. *C*, the facilitation of wind-up reversed upon wash out of ACPD. The stimulus intensity was adjusted in the different cases to reach the same initial membrane potential at the onset of the pulse. The same resting membrane potential was kept in all conditions by applying holding current. Fast synaptic transmission was blocked.

due to membrane depolarization, the ACPD-induced depolarization was compensated by injection of hyperpolarizing bias current. Furthermore, since ACPD usually produced a concomitant increase in input resistance, the strength of the depolarizing current pulse was adjusted to produce the same initial depolarization. The response recovered after washout of ACPD (Fig. 4*A c*). This cell did not show sustained after-discharges in control conditions even when stimulated from a depolarized holding membrane potential, a manoeuvre that facilitates the generation of after-discharges (Russo & Hounsgaard, 1996). In the presence of ACPD, however, even a brief stimulus could produce a long-lasting after-discharge (Fig. 4*B*). Moreover, the rate of increase in firing frequency in response to the same stimulus was dramatically increased by ACPD (Fig. 4*Ca* and *b*). The temporal evolution of the instantaneous firing frequency reflects the temporal evolution of the plateau potential during a stimulus (Russo & Hounsgaard, 1996). Comparison of the instantaneous frequency plots suggests that ACPD increased the rate of activation of the plateau potential (Fig. 4*Cc*).

The modulation of plateau properties by ACPD had important consequences for temporal integration. Figure 5 shows that ACPD potentiated wind-up. In control conditions, moderate wind-up of the response to repetitive depolarizing pulses (500 ms, 0·3 Hz) was observed (Fig. 5*A*). Addition of ACPD (50 μM) enhanced wind-up during the pulse (Fig. 5*B*) and a slow depolarizing potential accumulated between stimuli leading to an after-discharge. Note that the depolarization produced by ACPD was compensated for by injection of a hyperpolarizing holding current to keep the prestimulus resting membrane potential at the control value. Since ACPD increased the input resistance (from 83 to 125 MΩ), the strength of the depolarizing current pulse was adjusted to produce the same depolarization at the onset of the first depolarizing current pulse. After washout of ACPD the facilitation of wind-up disappeared (Fig. 5*C*).

The data shown above suggested that ACPD affected the excitability of plateau-generating neurones by changing the threshold and the time course of the plateau potential. This was more clearly observed in the presence of TTX (1 μM, $n = 11$). Figure 6 shows a cell with a weak plateau potential that activated around 20 mV above the resting membrane potential (Fig. 6*A a*, arrow). After addition of ACPD (50 μM) a powerful activation of the plateau potential was elicited with an initial depolarization of 15 mV (Fig. 6*A b*). In addition, the plateau potential decayed slowly after the pulse (Fig. 6*A b*). These observations may account for the increased firing rate and the after-discharges recorded in normal medium. In the presence of TTX the effect of the lower threshold and the slower decay of the plateau potential on wind-up generation was also obvious. Indeed, in the

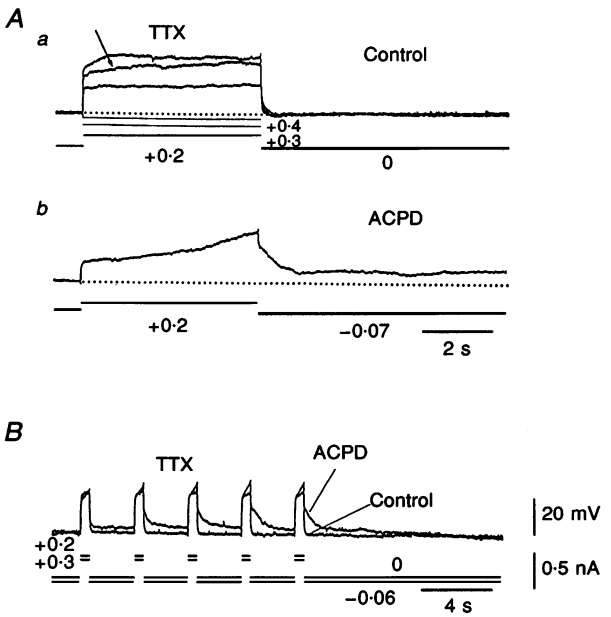

Figure 6. ACPD changed the threshold and time course of the plateau potential

All experiments were conducted in the presence of TTX (1 μM). *A*, superimposed traces of the response to depolarizing current pulses of three different amplitudes reveal the presence of a weak plateau potential that activated about 20 mV above resting potential (*a*, arrow). After the pulse the plateau potential decayed rapidly. In the presence of ACPD (50 μM) a strong activation of the plateau potential was obtained with just 15 mV of depolarization (*b*). The plateau potential decayed slowly after termination of the pulse. All stimuli applied at the same membrane potential. *B*, ACPD (50 μM) induced a wind-up-like activation of the plateau potential in response to a previously inefficient stimulation protocol. In the presence of ACPD the stimulation strength was lowered to produce the same initial depolarization as under control conditions, and a slight negative holding current was applied. All data were taken from the same cell.

presence of ACPD a previously insufficient stimulation protocol produced a prominent wind-up-like activation of the plateau potential (Fig. 6*B*).

All the effects induced by ACPD were reproduced by the selective mGluR1 agonist DHPG (50 μM, $n = 4$; not illustrated).

Effects of substance P on the excitability of plateau-generating neurones

Substance P also modulated the excitability by promoting plateau properties (Fig. 7). We noted that the increased excitability persisted during continued perfusion with medium containing substance P, in contrast to the transient nature of the substance P-induced depolarization. This made it possible to study the effects of substance P on the intrinsic responsiveness of plateau-generating neurones in the absence of depolarization. In the experiment illustrated in Fig. 7*A*, a moderate activation of the plateau potential in response to a depolarizing current pulse was suggested by the slow increase in firing frequency and by a tiny depolarizing after-potential (Fig. 7*A a*). In the presence of substance P (2 μM) the same stimulus produced a stronger response with a higher rate of firing frequency increase, reaching a high, steady level by the end of the pulse and followed by a prolonged after-discharge (Fig. 7*A b*). Note that the input resistance (100 MΩ) remained unchanged (Fig. 7*A*, inset). As for ACPD, the threshold for plateau-potential activation shifted to hyperpolarized membrane potentials as shown in the presence of TTX (1 μM, $n = 4$) (Fig. 7*Ba, b*). The superimposed responses in Fig. 7*B c* reveals that the same current pulse activated a plateau potential in the presence of substance P while a passive response was

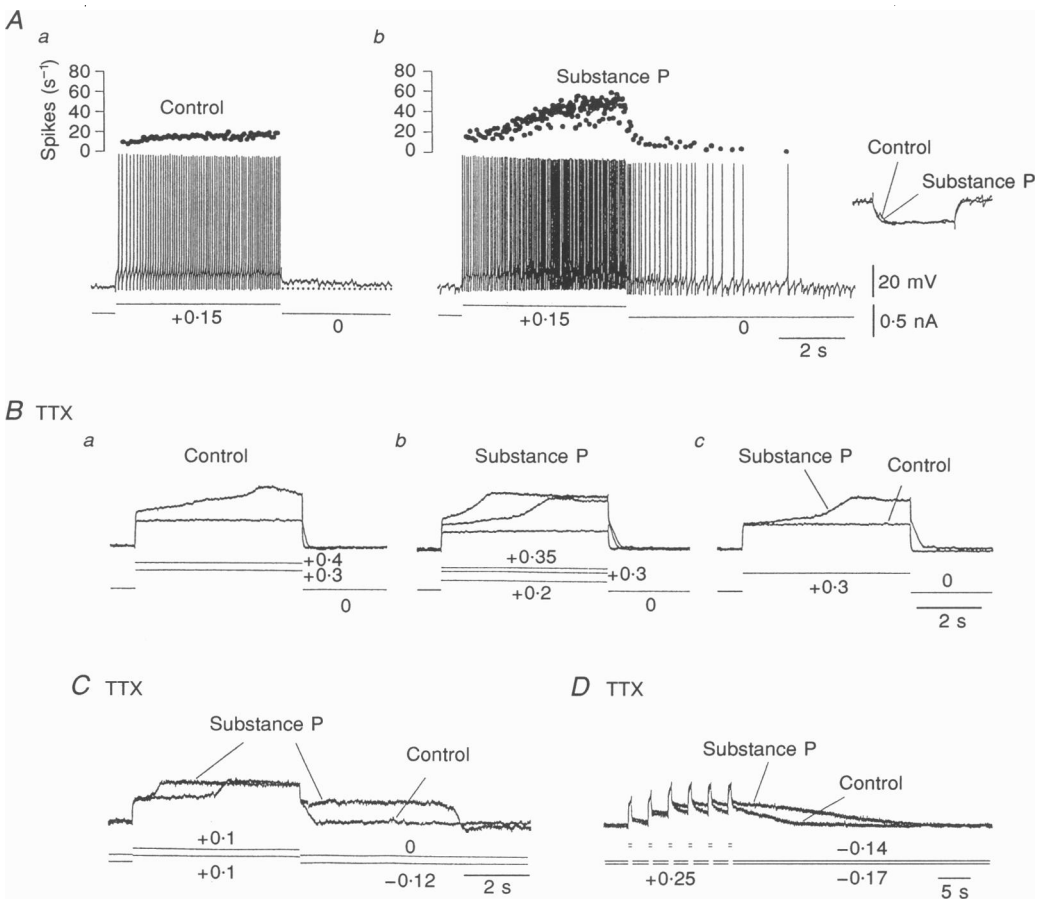

Figure 7. Modulation of plateau properties by substance P

A, increased excitability induced by substance P. In control conditions a slight increase in firing frequency suggested a mild activation of the plateau potential (*a*). Substance P (2 μM) potentiated the response, producing a higher rate of firing frequency increase during the pulse and facilitated the occurrence of after-discharges (*b*). Notice that in this cell the apparent input resistance (100 MΩ) did not change (inset). *B*, a clear plateau potential was elicited in the presence of TTX (1 μM) when the membrane potential was depolarized 21 mV from rest (*a*). Substance P lowered the threshold for plateau potential activation (*b*, 14 mV from rest). In *c*, two responses to the same stimulus before and after substance P are shown superimposed. *C*, in the presence of TTX (1 μM), substance P (2 μM) decreased the delay for activation and greatly prolonged the duration of the plateau potential. *D*, substance P (2 μM) potentiated the wind-up-like activation of the plateau potential. Data were taken from different cells.

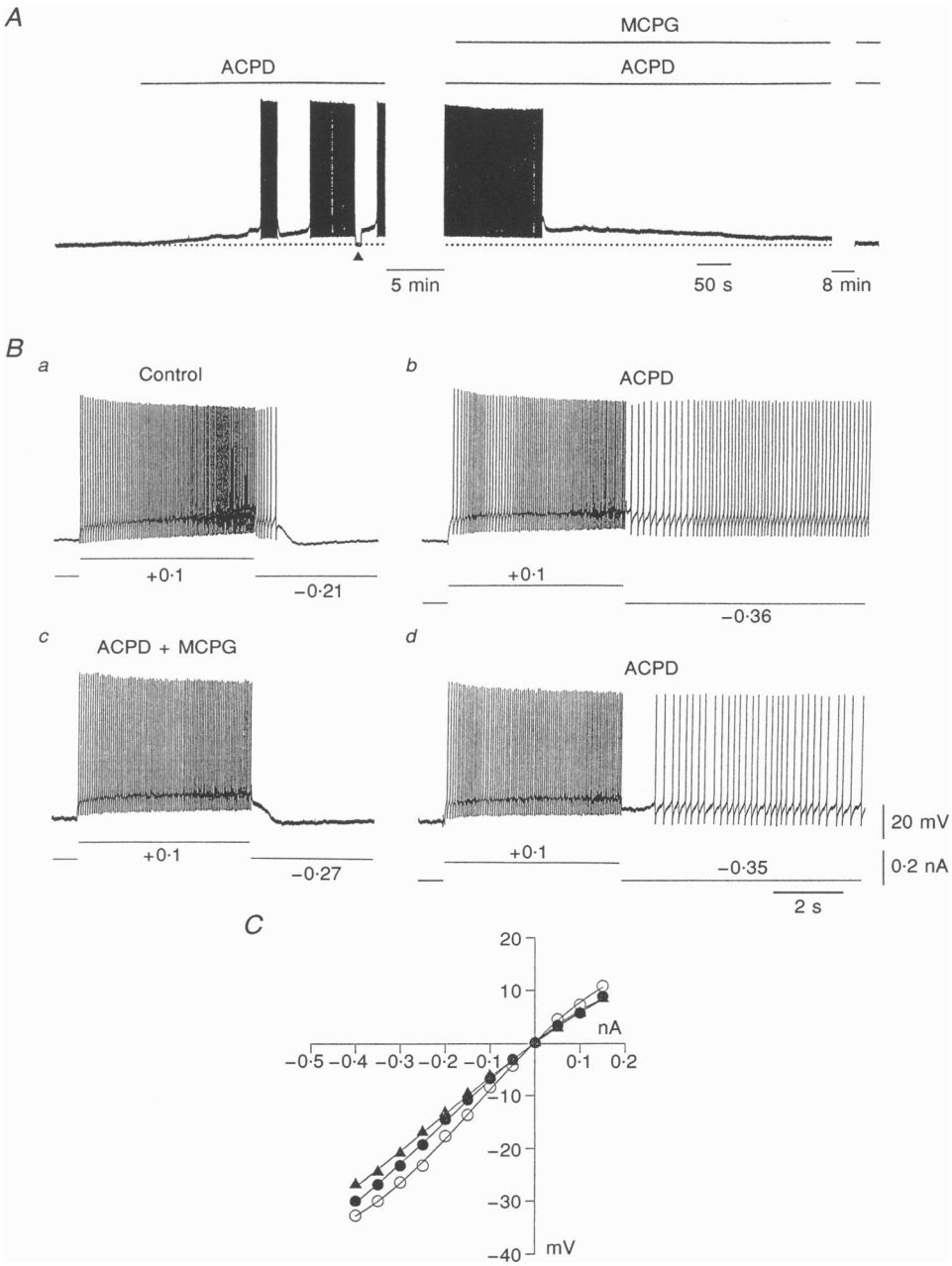

Figure 8. MCPG blocked the ACPD-induced effects

A, slow depolarization and spontaneous firing induced by ACPD (10 μM). The prolonged suppression of firing by a hyperpolarizing current pulse (7 s, ▲) suggested the activation of the plateau potential by the ACPD-induced depolarization. MCPG (1 mM) readily suppressed the sustained discharge induced by ACPD. The ACPD-induced depolarization was completely blocked after 13 min of MCPG application. *B*, a short after-discharge (*a*) strongly facilitated by 10 μM ACPD (*b*) was blocked by 1 mM MCPG (*c*). The promotion of the after-discharge by ACPD reappeared upon wash out of MCPG (*d*).. *C*, MCPG (1 mM) blocked the increase in apparent input resistance produced by ACPD (○). A slight decrease of input resistance with respect to control (●) was produced by combined ACPD and MCPG (▲). The lines represent 3rd-order regressions through data points. Data in *A* and *C* are from the same cell. Fast synaptic transmission was blocked in all cases.

obtained under control conditions despite an initially identical membrane potential trajectory (Fig. 7*Bc*). In addition, the decay of the plateau potential was slowed by substance P (Fig. 7*C*). These substance P-induced effects combined to facilitate the wind-up-like activation of the plateau potential (Fig. 7*D*).

Effects of mGluR and tachykinin receptor antagonists

Certain phenylglycine derivatives are specific antagonists of some mGluR receptor subtypes (Pin & Duvoisin, 1995). We found that MCPG (0·5–1 mM, $n = 5$) blocked all effects of ACPD. The experiment in Fig. 8 shows a cell in which ACPD (10 μM) produced a prominent slow depolarization leading to spontaneous firing (Fig. 8*A*). A brief hyperpolarizing current pulse stopped the firing (arrowhead) which resumed after about 20 s, suggesting that the plateau potential was activated by the ACPD-induced depolarization. As already mentioned, the ACPD-induced depolarization was stable during perfusion with the drug. However, 2 min after addition of MCPG (1 mM) the firing stopped (Fig. 8*A*). The cell gradually hyperpolarized to reach control values 13 min after addition of MCPG.

The modulation of the plateau properties by ACPD was also antagonized effectively by MCPG. Figure 8*B* shows that the prolonged after-discharge induced by 10 μM ACPD (Fig. 8*Bb*) was blocked by 1 mM MCPG (Fig. 8*Bc*). The facilitation of after-discharges by ACPD reappeared after washout of MCPG (Fig. 8*Bd*). Finally, MCPG also reversed the increase in input resistance produced by ACPD (Fig. 8*C*). Interestingly, in two out of five cells MCPG produced a slight decrease in excitability (Fig. 8*Bc*) and input resistance (Fig. 8*C*, from 68 to 64 MΩ) compared with control.

Since substance P is believed to act mainly at the NK-1 tachykinin receptor subtype (Otsuka & Yoshioka, 1993), we tested the effects of GR 82334 (5–10 μM, $n = 4$), a selective NK-1 receptor antagonist. We only assessed the effects of GR 82334 on the substance P-mediated modulation of the plateau properties because the depolarization induced by substance P was transient and the effects on input resistance were variable. The plateau potential of the cell shown in Fig. 9 was potentiated by substance P (2 μM), producing a stronger response during the stimulus and a prolonged after-discharge (Fig. 9*A* and *B*). The potentiation of the plateau potential was greatly reduced by 10 μM of GR 82334 (Fig. 9*C*, 4/4 cells). The selective NK-2 antagonist MEN 10376 (2 μM, $n = 3$) had no effect on the promotion of plateau properties induced by substance P.

The mGluR and NK-1 receptor antagonists also reduced the modulation of plateau properties induced by tetanic DR stimulation. These results are summarized in Fig. 10 which shows a neurone in which DR stimulation lowered the threshold for plateau potential activation (Fig. 10*A a* and *b*).

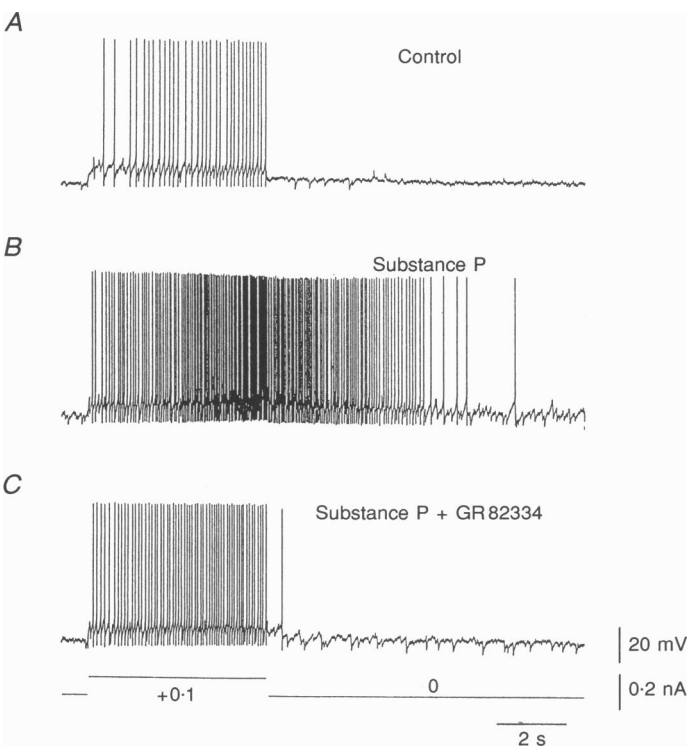

Figure 9. GR 82334 blocked the potentiation of plateau properties produced by substance P

The mild plateau properties observed in control conditions (*A*) were strongly potentiated by substance P (*B*, 2 μM). A clear reduction of the substance P-induced effect was observed after addition of the selective NK-1 antagonist GR 82334 (*C*, 10 μM).

This effect was reversibly blocked by 1 mM MCPG (Fig. 10*B* and *C*; 5/5 cells). MCPG also reduced the slow depolarization induced by prolonged stimulation of the DR (2/3 cells). In three out of five neurones GR82334 (10 μM) antagonized the facilitation of the plateau potential induced by tetanic DR stimulation (Fig. 10*D* and *E*). However, GR82334 did not reduce the slow depolarization (3/3 cells).

Effects of ACPD and substance P on non-plateau-generating neurones

To test whether ACPD or substance P could induce plateau properties in non-plateau-generating neurones in the dorsal horn, we evaluated the effects on cells in which no signs of plateau potential were observed.

ACPD (50 μM) induced a similar slow depolarization and increase in input resistance as in plateau-generating neurones (not illustrated). The excitability was also increased by ACPD (4/8 cells). As shown in Fig. 11*A*, ACPD increased the firing frequency of APs in response to the same stimulus in phasic (Fig. 11*A a* and *b*) and tonic (Fig. 11*A c*) neurones. Note, however, that the firing pattern remained unchanged.

Substance P increased the excitability of six out of eleven non-plateau neurones (Fig. 11*Ba* and *b*) but in a similar manner to ACPD was unable to induce plateau properties.

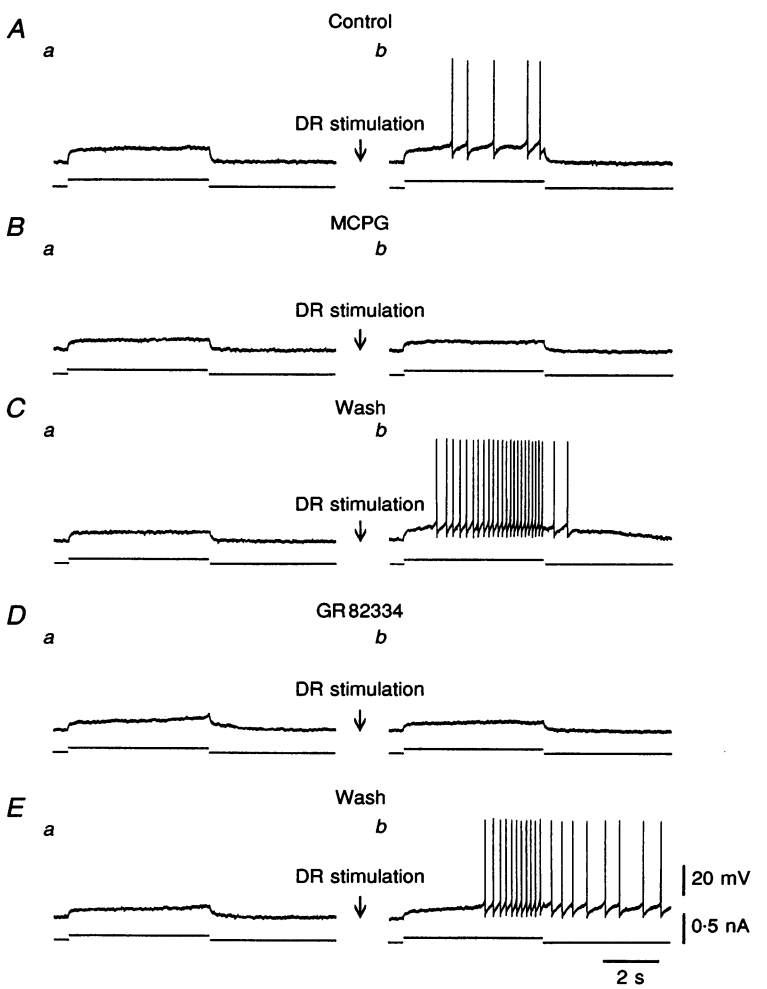

Figure 10. MCPG and GR 82334 antagonized the facilitation of plateau properties induced by DR stimulation

A, in the intensity of a depolarizing current pulse was adjusted just below the level that would produce plateau potential activation (*a*). After DR stimulation (30 μA at 10 Hz during 0·5 min) the previously subthreshold pulse produced plateau potential activation (*b*) as indicated by the delayed firing of the cell. *B*, the same protocol as used in *A* but in the presence of 1 mM MCPG failed to lower the threshold for plateau potential activation (*Ba* and *b*). *C*, the facilitatory effect was fully recovered upon washout of MCPG (*Ca* and *b*). *D*, addition of GR82334 (10 μM) to the superfusate also reduced the promotion of plateau properties induced by stimulation of the DR (*Da* and *b*). As shown in *E* this was reversed upon washout of the drug (*Ea* and *b*). The membrane potential before the depolarizing current pulse was −58 mV in all cases. Fast synaptic transmission was blocked. All data were taken from the same cell.

DISCUSSION

Neuromodulation is believed to play a critical role in the plastic changes induced by nociceptive inputs (Dubner & Ruda, 1992; McMahon *et al.* 1993; Urban *et al.* 1994). Our study shows that the intrinsic response properties of plateau neurones in the dorsal horn are modulated by primary afferent activity and by transmitters known to be released in the spinal cord. The modulation affects both the magnitude and temporal integration of postsynaptic responses. The evidence suggests that the L-type Ca^{2+} channels underlying plateau potentials are targets for modulation induced by activation of metabotropic glutamate receptors and NK-1 receptors. These findings suggest that modulation of postsynaptic response properties contributes to the plasticity of sensory processing in the dorsal horn.

Modulation of plateau-generating neurones by primary afferent stimulation

The ability of primary afferents to modulate the response of dorsal horn neurones may arise both from activity-dependent changes in the postsynaptic cell (Dubner & Ruda,

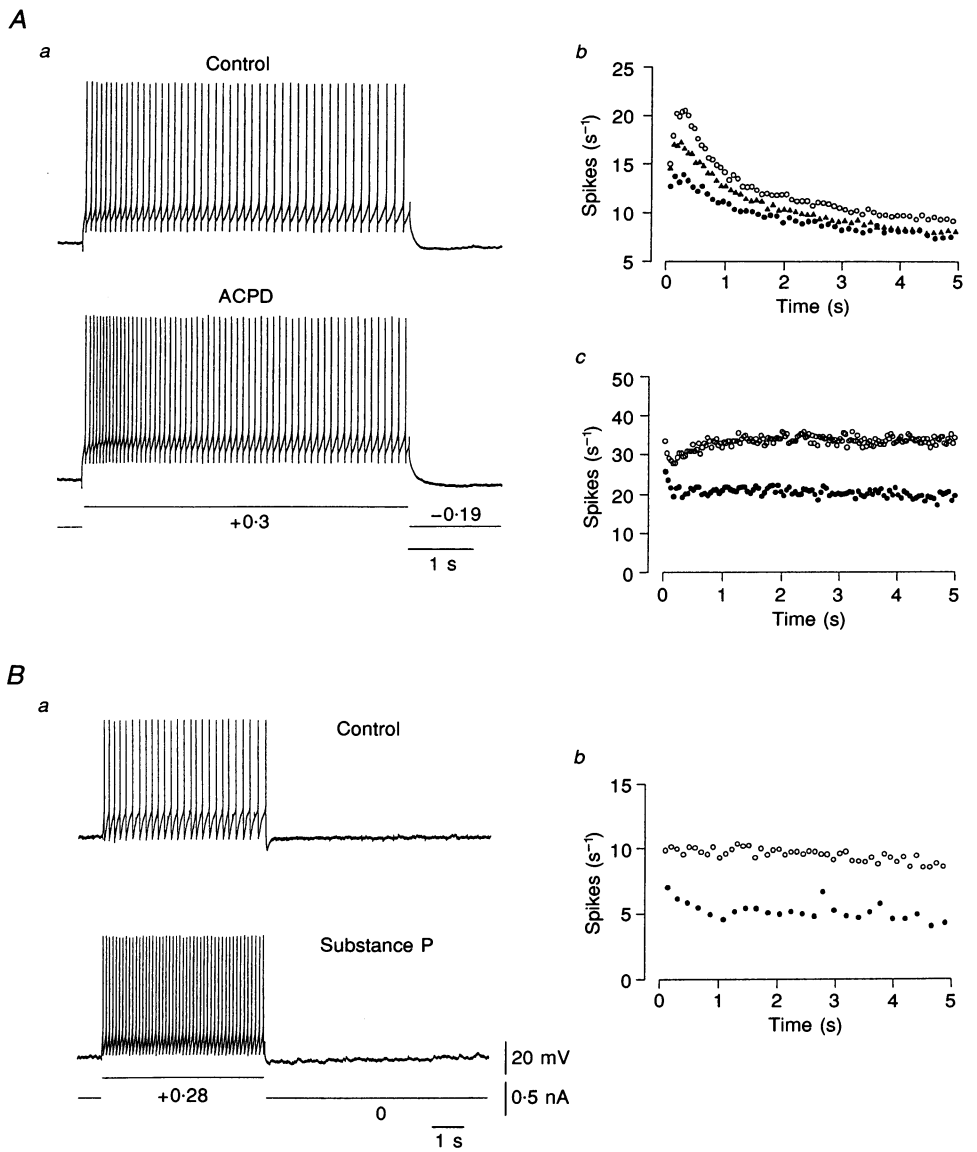

Figure 11. Effects of ACPD and substance P on non-plateau-generating neurones

A, ACPD (50 μM) increased the excitability of a cell displaying a marked firing frequency adaptation in response to a depolarizing current pulse. However, the discharge pattern remained unchanged (*a* and *b*: Control, ●; ACPD, ○; Wash, ▲). A similar effect of ACPD (50 μM) was observed in another neurone with a tonic pattern of discharge (*c*: symbols as *A b*). *B*, substance P increased the response of a phasic-tonic neurone to a depolarizing current pulse (*a*). Comparison of the IFPs reveals a slight decrease of the initial adaptation (*b*: Control, ●; Substance P, ○). Stimuli before and after the addition of the drug were applied at the same membrane potential.

1992) and by the action of neuromodulators (Urban *et al.* 1994). Our experimental design excludes the possibility that the depolarization of dorsal horn neurones and the enhancement of plateau potentials and wind-up following intense primary afferent activity is secondary to postsynaptic spike firing or changes in polysynaptic pathways. This implies that the effects studied are produced directly by modulatory substances released by primary afferent terminals.

As in mammals (Jessell & Dodd, 1989), glutamate is likely to be released by primary afferents in the turtle since the fast synaptic transmission was greatly reduced by AMPA and NMDA receptor antagonists. Another possible transmitter released by DR stimulation is substance P which is contained in thin, unmyelinated afferents, both in mammals (Otsuka & Yoshioka, 1993) and turtles (Luthman, Fernández, Radmilovich & Trujillo-Cenóz, 1991). Substance P is known to be released by nociceptive afferents (Duggan, Hendry, Morton, Hutchinson & Zhao, 1988) and to mediate slow synaptic actions (Urban & Randic, 1984; De Koninck & Henry, 1991). The modulatory effects of primary afferent activity were mimicked remarkably well by activation of substance P receptors and mGluRs and were antagonized by selective blockers for mGluR and NK-1 receptor. In some cells the facilitation of plateau properties by DR stimulation was substantially reduced by both MCPG and GR 82334. The possible co-operation between mGluR and NK-1 receptors suggested by this surprising finding, has not yet been explored experimentally.

These results show that glutamate and substance P released from primary afferents play an important part in the regulation of postsynaptic properties in plateau-generating neurones of the dorsal horn.

Depolarization induced by ACPD and substance P

The effects of ACPD and substance P on the membrane potential were essentially similar with the exception that the substance P-induced depolarization was transient. As in the hippocampus (Charpak, Gähwiler, Do & Knöpfel, 1990) the ACPD-induced depolarization was associated with an increase in input resistance. In the hippocampus, reduction of three K^+ conductances have been suggested to mediate the ACPD-induced depolarization, an M-like current, a calcium-dependent K^+ current (Charpak *et al.* 1990) and a K^+ leak conductance (Guérinau, Gähwiler & Gerber, 1994). It is possible that reduction of a K^+ leak conductance mediates the depolarization observed in our study since ACPD induced a symmetrical shift in the current–voltage curve.

The substance P-induced depolarization observed in the present study was similar to the slow depolarization described in mammalian dorsal horn neurones (Nowak & MacDonald, 1982; Murase & Randic, 1984). As in mammals, the depolarization may be mediated by a reduction of a K^+ conductance since it was associated with an increase in input resistance in most cells. However, in other cells no change or even a decrease in input resistance was observed. Similar observations have been reported in cat dorsal horn neurones (Zieglgänsberger & Tulloch, 1979). This variability may be related to the fact that several ionic conductances are affected by tachykinins in dorsal horn neurones (Murase *et al.* 1989).

Modulation of plateau properties

ACPD and substance P also increased excitability by facilitating the plateau potential. The decreased threshold for the activation of the plateau potential may be due to blockade of a counteracting conductance or by facilitation of the plateau potential itself. In the hippocampus ACPD increases excitability by blockade of a Ca^{2+}-dependent K^+ conductance $g_{K(Ca)}$ (Charpak *et al.* 1990). This is unlikely to be a major mechanism in plateau-generating neurones since no change in spike after-potentials was observed (R. E. Russo, F. Nagy & J. Hounsgaard, unpublished observations). As already mentioned, the steeper current–voltage relationship in the presence of ACPD and substance P suggested a block of a leak conductance. This might change the delicate balance of different membrane conductances resulting in an apparent reduction in threshold for activation of the plateau potential. However, our data strongly suggest a direct action on L-type Ca^{2+} channels since ACPD and substance P produced a powerful facilitation of the plateau potential even when the input resistance remained unchanged. Interestingly, the facilitation of the plateau potential by the dihydropyridine agonist Bay K 8644 was also associated with a decrease in threshold for plateau potential activation (Russo & Hounsgaard, 1996). Furthermore, voltage-clamp studies in mammalian dorsal horn neurones showed that substance P potentiated a slow, voltage-dependent Ca^{2+} current which activated close to the resting membrane potential, was reduced by verapamil and potentiated by Bay K 8644 (Murase, Ryu & Randic, 1986, 1989).

The finding that ACPD facilitated the plateau potential is remarkable since several studies have reported inhibition of L-type Ca^{2+} currents by activation of mGluRs (Sayer, Schwindt & Crill, 1992; Chavis, Shinozaki, Bockaert & Fagni, 1994*a*). However, potentiation of L-type currents was found in cerebellar granule cells (Chavis, Nooney, Bockaert, Fagni, Feltz & Bossu, 1994*b*).

Neuromodulation and nociceptive mechanisms

A role for substance P in nociceptive mechanisms is widely hypothesized (Willis & Coggeshall, 1991; Zieglgänsberger & Tölle, 1993; McMahon *et al.* 1993). Substance P is released by noxious but not by non-noxious stimuli (Duggan *et al.* 1988) and its biosynthesis in primary sensory neurones is increased by nociceptive stimuli (Otsuka & Yoshioka, 1993). Furthermore, intrathecal application of substance P produces pain-related behaviour whereas injection of substance P antagonists has an analgesic effect (Otsuka & Yoshioka, 1993). Substance P has a preferential affinity for the NK-1 tackychinin receptor subtype (Guard & Watson, 1991)

which is widely distributed in the dorsal horn. In agreement with this, the potentiation of plateau potentials induced by substance P was blocked by the selective NK-1 antagonist GR 82334 while MEN 10376 (a selective NK-2 antagonist) had no effect. Interestingly, recent studies have shown that CP 96345 (a non-peptide NK-1 receptor antagonist) has anti-nociceptive effects in the formalin and capsaicin tests (Sakurada, Katsumata, Yogo, Tan-No, Sakurada & Kisara, 1993). Although this drug also has antagonist effects on Ca^{2+} channels (Schmidt, McLean & Heym, 1992) the involvement of the NK-1 receptor in nociceptive mechanisms is supported by results based on the specific antagonist GR 82334 (Dougherty, Palecek, Paleckova & Willis, 1994).

Metabotropic glutamate receptors are thought to play a role in modulation of synaptic transmission and neuronal excitability in the brain and contribute to regulate neuronal networks (Pin & Duvoisin, 1995) including nociceptive circuits in the spinal cord (Meller, Dykstra & Gebhart, 1993; Young, Fleetwood-Walker, Mitchell & Munro, 1994; Boxall, Thompson, Dray, Dickenson & Urban, 1996). In our study, all the effects induced by ACPD on plateau-generating neurones were blocked by MCPG which is a competitive antagonist for metabotropic receptors. ACPD and MCPG are mainly active on group I and II metabotropic receptors (Pin & Duvoisin, 1995). The fact that DHPG produced the same effects as ACPD suggests that the enhancement of plateau properties is mediated by mGluR1. This is in agreement with the facilitation of L-type Ca^{2+} currents produced by mGluR1 in cerebellar granule cells (Chavis *et al.* 1994*b*).

Both substance P (Rusin, Bleakman, Chard, Randic & Miller, 1993) and ACPD (Cerne & Randic, 1992) potentiate ionotropic glutamate responses in dorsal horn neurones, which may contribute to their role in nociceptive mechanisms (Urban *et al.* 1994). Our study suggests that modulation of the plateau properties also plays a role. The lower threshold for plateau activation enabled the cells to respond to previously subthreshold stimuli and to produce stronger responses to suprathreshold stimuli. This may play a part in allodynia and hyperalgesia. In addition, modulation of the plateau potential by mGluRs and NK-1 receptors facilitated wind-up and promoted after-discharges, which are phenomena thought to be early events in central sensitization to pain (McMahon *et al.* 1993).

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