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Metabotropic glutamate receptor-mediated excitation and inhibition of sympathetic preganglionic neurones

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Abstract

The effects of metabotropic glutamate receptor (mGluR) subtype selective compounds on the excitability of sympathetic preganglionic neurones (SPNs) were investigated. Non-selective mGluR agonists (1*S*,3*R*)-aminocyclopentane-1,3-dicarboxylic acid and (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine, induced dose-dependent depolarisations in 96 and 75% of SPNs, respectively and hyperpolarisations in 2 and 21% of SPNs. Both agonists could induce subthreshold membrane potential oscillations in previously non-oscillating SPNs and either increased or reduced the frequency of spontaneously occurring oscillations. A selective group I mGluR agonist, 3,5-dihydroxyphenylglycine, depolarised all SPNs tested, induced oscillations in membrane potential of otherwise non-oscillating SPNs and increased the frequency of spontaneous oscillations. Agonists with selectivity for group II mGluRs (1*S*, 3*S*)-aminocyclopentane-1,3-dicarboxylic acid and (*S*)-4-carboxy-3-hydroxy-phenylglycine ((*S*)-4C3HPG) did not induce depolarising responses. However (*S*)-4C3HPG induced hyperpolarising responses associated with a reduction in the frequency of spontaneous oscillations in two of six SPNs tested. Depolarising and hyperpolarising responses were maintained in the presence of tetrodotoxin indicating a direct action of the agonists upon SPNs. In individual SPNs responses of opposite polarity could be induced from the same initial membrane potential using different agonists, indicating that the opposing responses involved different ionic mechanisms. The broad spectrum mGluR antagonist (*S*)-α-methyl-4-carboxyphenylglycine and the selective group I mGluR antagonist (*S*)-4-carboxyphenylglycine reversibly depressed mGluR agonist induced depolarisations. These results indicate that SPNs express two mGluR populations with opposing actions on neuronal excitability: group I mGluRs depolarise SPNs and can drive oscillatory membrane potential activity; a minority of SPNs express group II mGluRs which mediate membrane hyperpolarisations and reduce the frequency of membrane potential oscillations.

Keywords: Metabotropic glutamate receptors; Phenylglycines; Sympathetic control; Preganglionic neurone; Oscillations

1. Introduction

Sympathetic preganglionic neurones (SPNs) form the final central site for integration of information leading to the generation of sympathetic output. The output of SPNs is determined by their intrinsic membrane properties, by electrotonic coupling between adjacent SPNs and by synaptic inputs from spinal and supraspinal neurones (Coote, 1988; Cabot, 1990; Logan et al., 1996). Glutamatergic synapses make up from half to two thirds of the synapses onto SPNs (Llewellyn-Smith et al., 1992, 1995) and inputs from brainstem cardiovascular control areas to SPNs utilise glutamate as a neurotransmitter (Minson et al., 1991; Morrison et al., 1991; Deuchars et al., 1995). Glutamatergic synaptic inputs are therefore likely to have a fundamental role in the generation of sympathetic output by SPNs. Characterisation of the identity and cellular actions of glutamate receptors expressed by SPNs is necessary in order to determine their involvement in this process. Glutamate receptors are commonly divided into ionotropic and metabotropic groups. Numerous studies have examined the pharmacology of ionotropic glutamate receptors (iGluR) expressed by SPNs: agonists for N-methyl-D-aspartate (NMDA) and non-NMDA iGluRs induce depolarisations or inward currents which can be blocked by selective antagonists (Spanswick and

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Logan, 1990; Inokuchi et al., 1992; Krupp and Feltz, 1995) and fast excitatory synaptic inputs to SPNs have components which are blocked by non-NMDA and NMDA receptor antagonists (Inokuchi et al., 1992; Deuchars et al., 1995; Krupp and Feltz, 1995). Considerably less is known about the properties of metabotropic glutamate receptors (mGluRs) expressed by SPNs. In a previous study mGluR agonists were shown to have excitatory actions on SPNs, including membrane depolarisation, induction of subthreshold membrane potential oscillations and modification of the action potential waveform (Spanswick et al., 1995).

The mGluRs are a family of seven transmembrane domain G-protein coupled receptors whose activation is coupled to various second messenger pathways (Pin and Duvoisin, 1995). The eight cloned mGluRs (mGluR1–mGluR8) can be divided into three groups on the basis of their sequence homologies and signal transduction properties (Nakanishi, 1992; Pin and Duvoisin, 1995). In expression systems receptors from group I (mGluR1 and mGluR5) stimulate phosphoinositide hydrolysis; whereas receptors from group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7 and mGluR8) inhibit adenylyl cyclase activity. Each group of cloned mGluRs has a distinct pharmacological profile, enabling the subgroup identity of receptors mediating mGluR responses observed *in situ* to be determined using pharmacological tools (Watkins and Collingridge, 1994; Pin and Duvoisin, 1995).

The mGluR-mediated excitation of SPNs described in our previous study was induced by the agonists (1*S*,3*R*)-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD) and quisqualate, suggesting the involvement of a group I mGluR (Spanswick et al., 1995). However, both agonists are active at more than one class of mGluR and quisqualate also at ionotropic glutamate receptors (Pin et al., 1994; Saugstad et al., 1997). The group III selective agonist L(+)-2-amino-4-phosphonobutyric acid (L-AP4) was also reported to depolarise SPNs at concentrations of 0.1–1 mM (Spanswick et al., 1995). The actions on SPNs of agonists with selectivity for group I or group II mGluRs have not previously been examined. Here, during whole-cell patch-clamp recordings from SPNs, we have examined the actions of pharmacological tools with selectivity between mGluRs subtypes in order to identify the mGluRs expressed by SPNs and to determine their cellular actions. In addition we have attempted to identify agonists and antagonists which may be useful for clarification of the physiological role of these receptors in the generation of sympathetic output by SPNs. Some of this work has been reported previously in abstract form (Nolan and Logan, 1994, 1996).

2. Methods

The experimental procedures used for preparation of spinal cord slices and for obtaining whole-cell recordings from SPNs have been described in detail previously (Spanswick and Logan, 1990; Pickering et al., 1991; Logan et al., 1996). Sprague-Dawley rats (7–16 days old) were anaesthetised with enflurane (Abbott), decapitated, the spinal cord removed and 300–500 μm transverse thoracolumbar slices cut using a vibratome. The slices were maintained in artificial cerebrospinal fluid (aCSF) of composition (mM): NaCl 127, KCl, 1.9, KH_2PO_4 1.2, $CaCl_2$ 2.4, $MgSO_4$ 1.3, $NaHCO_3$ 26, D-Glucose 10, equilibrated with 95% $O_2$5% CO_2. Slices were used for recording between 1 and 24 h after preparation. Slices were maintained before and during recording at room temperature (18–22°C).

Whole-cell recordings were made in current-clamp mode from neurones in the intermediolateral column as described previously (Pickering et al., 1991). During recording, slices were held in place between two grids, in a chamber (volume 2.5 ml) continuously perfused with aCSF at a rate of 4–8 ml min^{-1}. The patch electrodes had resistance 3–12 MΩ and were filled with the following solution (mM): potassium gluconate 130, KCl 10, $MgCl_2$ 2, $CaCl_2$ 1, EGTA-Na 1, HEPES 10, Na_2ATP 2 and either Lucifer yellow 2 or biocytin 5, pH adjusted to 7.4 with KOH, osmolarity adjusted to 315 mOsm with sucrose. Recordings were made using a List EPC7 (List-medical) amplifier. The current and voltage output from the amplifier were displayed on-line on an oscilloscope (Gould DSO 1602) and chart recorder (Electromed, Multipulse 2) and digitised (Sony, PCM-701ES) and stored on videotape (Sony, SL-F25UB) for later playback and analysis. Neurones with stable resting membrane potentials and action potentials which overshot 0 mV were identified as healthy and suitable for recording. SPNs were identified during recording by their characteristic electrophysiological properties (Spanswick and Logan, 1990; Pickering et al., 1991).

All drugs were applied to the slice by inclusion in the perfusate. Examination of the time-course of membrane potential changes caused by perfusion of aCSF containing elevated or reduced concentrations of K^+, showed that at the perfusion rates used, equilibration took 3–4 min. In order to facilitate the rapid application and removal of agonists, they were applied in boluses for relatively short time periods (2–120 s) and at relatively high concentrations. As the agonist was not allowed to equilibrate in the bath, changes in either the duration of perfusion or the agonist concentration could be used to vary the concentration of agonist reaching the slice. However, the agonist concentration within the slice was unknown and may have been considerably lower than that added to the perfusate. For experiments where more than one agonist was applied to the same neu-

rone, the order of agonist application was varied between experiments to ensure that the observed responses did not depend upon this order. All antagonists were perfused for at least 5 min, to allow equilibration in the slice, prior to testing their effects on agonist responses.

During the majority of experiments agonists were perfused onto neurones held at their resting membrane potential (no applied current). During recording from neurones which fired spontaneous action potentials the actions of agonists on spontaneous activity (no applied current) and on membrane potential during injection of a constant negative current sufficient to hyperpolarise the cell below action potential threshold (5–50 pA) were both usually examined. When comparing multiple responses from the same neurone each bolus of agonist was applied with the neurone at the same initial resting membrane potential (if necessary the membrane potential was adjusted by injection of appropriate polarising current). Drug-induced membrane polarisations were identified as such when a reversible change in membrane potential of ≥ 2 mV was observed following agonist perfusion. For determination of input resistance changes occurring during agonist responses, the agonist induced membrane polarisation was opposed by manual current injection, so as to maintain the membrane potential at its value prior to agonist application and the input resistance was calculated from the membrane potential response to regularly applied negative current steps (amplitude 10–40 pA, duration 500–1000 ms). Statistical analysis was carried out using StatView2 (Abacus Concepts). Mean values are presented as mean $\pm$ standard deviation.

Following the majority of experiments an examination of neuronal morphology was carried out so as to confirm the identity of the recorded neurones. The procedures used for processing of the slices have been described in detail previously (Pickering et al., 1991; Logan et al., 1996). Slices were fixed with 2–4% paraformaldehyde in 0.1 M phosphate buffer for 1–2 h. Neurones filled with Lucifer yellow were cleared in dimethyl sulphoxide and viewed with an epifluorescence microscope. Neurones filled with biocytin were processed using a Vecstatin ABC kit (Vector Labs) and mounted on coverslips before viewing (see Logan et al., 1996). All of the electrophysiologically identified SPNs examined had morphological properties consistent with previous descriptions of SPNs (Forehand, 1990; Pickering et al., 1991).

The following drugs were used: (1*S*,3*R*)-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD), (1*S*,3*S*)-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*S*)-ACPD), L(+)-2-amino-4-phosphonobutyric acid (L-AP4), D(-)-2-amino-5-phosphonopentanoic acid (D-APV), (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine (L-CCG-I), (*S*)-4-carboxy-3-hydroxy-phenylglycine ((*S*)-4C3HPG) (*S*)-4-carboxyphenylglycine ((*S*)-4CPG), (*RS*)-2-chloro-5-hydroxyphenylglycine (CHPG), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), (2*S*,2′,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV), 3,5-dihydroxyphenylglycine (DHPG) (*S*)-α-methyl-4-carboxyphenylglycine ((+)-MCPG), 6-nitro-7-suplhamoylbenzo(f)quinoxaline-2,3-dione (NBQX) and quisqualate from Tocris Cookson (Bristol, UK) and tetrodotoxin (TTX) from Sigma (UK).

3. Results

The results described were obtained during whole-cell current-clamp recordings from over 150 SPNs. The electrophysiological properties of the SPNs were similar to those described previously (Pickering et al., 1991). The SPNs had resting membrane potentials between -45 and -80 mV, action potential amplitudes between 50 and 95 mV and action potential durations of 2–14 ms. The input resistance, calculated for 32 SPNs, ranged from 257 to 1726 MΩ (mean 651 ± 365 MΩ) and the membrane time constant, calculated for 22 SPNs, was between 38 and 220 ms (mean 102 ± 52 ms).

3.1. Effects of mGluR agonists on the membrane potential of SPNs

The previous report that (1S,3R)-ACPD and quisqualate depolarise SPNs (Spanswick et al., 1995) was reproduced in this study. Perfusion of (1*S*,3*R*)-ACPD (5–200 μM, for 2–120 s) depolarised 137 of 143 SPNs (Fig. 1). The amplitude of the depolarisation was dose-dependent (Fig. 1) and was maintained in the presence of TTX (500 nM, $n = 13$). Hyperpolarising responses to (1*S*,3*R*)-ACPD were observed during recordings from 3 SPNs. A further three SPNs did not show any apparent response to perfusion of (1*S*,3*R*)-ACPD. Perfusion of the L isomer of quisqualate, L-quisqualate (1–5 μM, 2–40 s), in the presence of iGluR antagonists (20 μM CNQX or 5 μM NBQX) also depolarised SPNs ($n = 4$). In this study neither (1*S*,3*R*)-ACPD or any of the compounds described below had any consistent effects on the properties of the action potential or input resistance (data not shown). In order to determine the pharmacological profile of the mGluRs mediating the depolarisation and hyperpolarisation of SPNs, responses to (1*S*,3*R*)-ACPD were compared to agonists that are reported to show selectivity for specific mGluRs.

DHPG has been reported to act as a selective agonist for IP_3 linked mGluRs and as an agonist at cloned, group I mGluRs (Ito et al., 1992; Schoepp et al., 1994; Gereau and Conn, 1995). Perfusion of DHPG (25–100 μM, 2–60 s) depolarised all of the SPNs tested ($n = 41$, Fig. 1A). Responses of silent SPNs to DHPG were

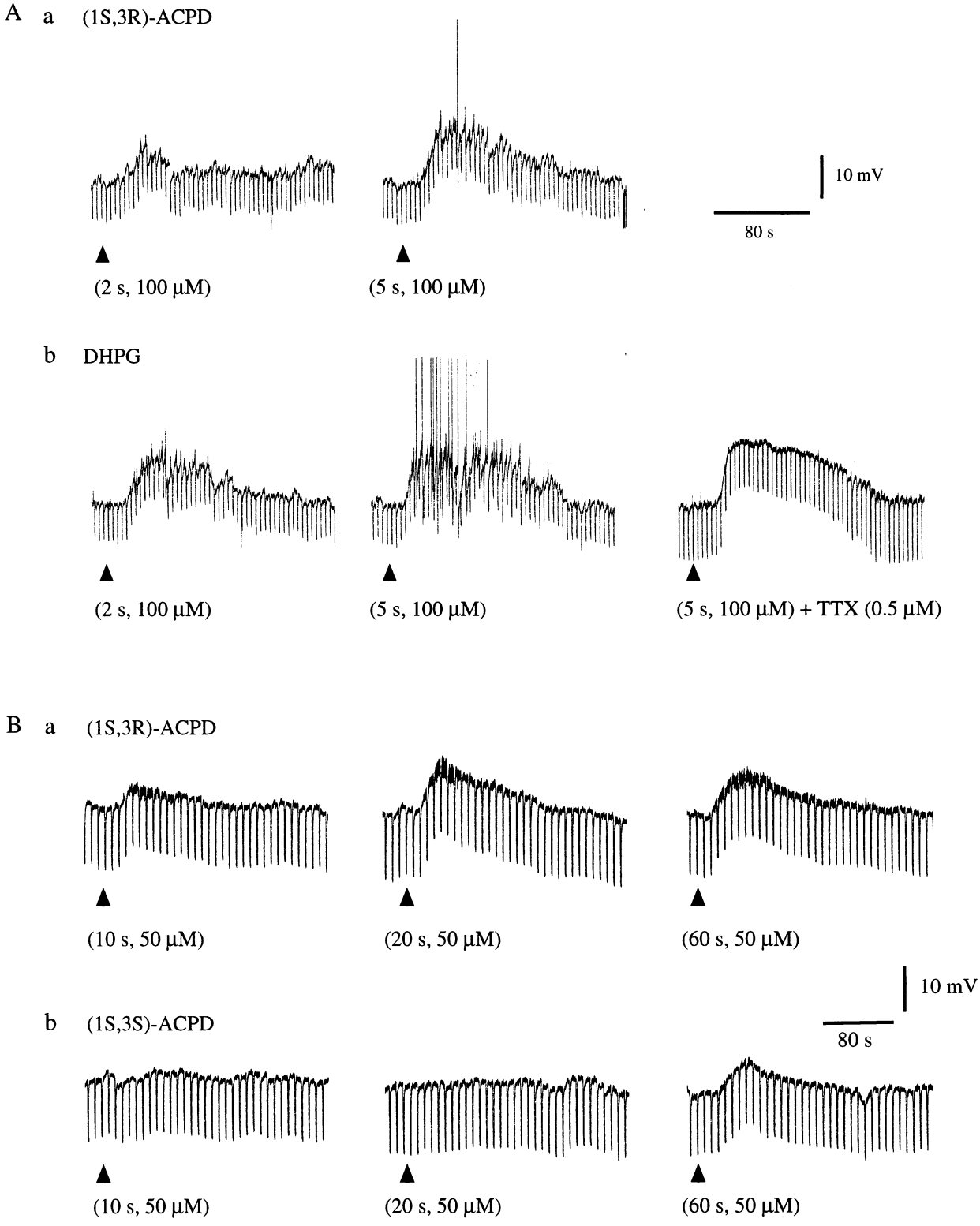

Fig. 1. Agonist pharmacology of mGluR agonist induced depolarisation of SPNs. (A) Depolarisations following perfusion of identical doses of (1*S*,3*R*)-ACPD (a) and DHPG (b) onto the same SPN (estimated resting membrane potential (Em) −60 mV). Depolarising responses to DHPG were maintained in the presence of 500 nM TTX indicating a direct action on SPNs. (B) Comparisons of responses of an SPN (Em −60 mV) to (1*S*,3*R*)-ACPD (a) and (1*S*,3*S*)-ACPD (b). (1*S*,3*S*)-ACPD at doses equivalent to sub-maximal doses of (1*S*,3*R*)-ACPD had no effect on membrane potential or input resistance, whereas at a dose equivalent to a supramaximal dose of (1*S*,3*R*)-ACPD; (1*S*,3*S*)-ACPD induced a depolarisation. In this and subsequent figures regular negative membrane potential deflections correspond to injection of negative rectangular waveform current pulses and give an indication of changes in input resistance. Arrows indicate the start of agonist perfusion, the duration of perfusion and concentration of agonist are stated in brackets.

often accompanied by action potential firing and those of active SPNs by an increase in the frequency of action potential firing. The depolarisation induced by DHPG was dose-dependent ($n = 12/12$, Fig. 1A) and insensitive to TTX (500 nM, $n = 2$, Fig. 1Ab). Comparison with the effects of (1*S*,3*R*)-ACPD on membrane potential showed that DHPG caused qualitatively similar effects (19 of 19 cells) and that when responses of the same SPN to two or more identical doses of each agonist were compared DHPG appeared to be more potent ($n = 6/6$, Fig. 1A). Inhibitory responses to DHPG were not observed, even from neurones that were hyperpolarised by other mGluR agonists ($n = 3$).

Group I mGluRs may be differentiated pharmacologically using the agonist CHPG, which is active at mGluR5a but not mGluR1α (Doherty et al., 1997). Perfusion of CHPG (500–1000 μM, 60–120 s) induced dose-dependent depolarisations in all of the SPNs tested ($n = 5/5$; data not shown). Responses to CHPG were observed in the presence of iGluR antagonists NBQX (2 μM) and D-APV (20 μM) indicating that CHPG was not acting via AMPA, kainate or NMDA receptors.

The (1*S*,3*S*)-enantiomer of ACPD has previously been shown using ventral root recordings to depress synaptic excitation of motor neurones without affecting baseline potentials, unlike (1*S*,3*R*)-ACPD which depresses synaptic transmission and also induces a depolarisation (Pook et al., 1992). This suggests that (1*S*,3*S*)-ACPD may have some selectivity for group II mGluRs, although at high concentrations it also induces group I mediated responses (Davies et al., 1995). Comparisons were made between dose-dependent depolarisations induced by (1*S*,3*R*)-ACPD and responses to perfusion of the same doses of (1*S*,3*S*)-ACPD (50 μM, 5–60 s) in four neurones (Fig. 1B). Perfusion of (1*S*,3*S*)-ACPD at the same concentration and for the same duration as sub-maximal doses of (1*S*,3*R*)-ACPD had no apparent effect on the membrane potential or input resistance of SPNs (Fig. 1Bb). One SPN was depolarised in response to (1*S*,3*S*)-ACPD at a dose that was supramaximal for the (1*S*,3*R*)-ACPD induced depolarisation (Fig. 1B).

L-CCG-I is a broad spectrum mGluR agonist that has some selectivity for group II mGluRs (Hayashi et al., 1992; Nakanishi, 1992). L-CCG-I (5–50 μM, 1–60 s) depolarised 18 of 24 SPNs (Fig. 2A). Depolarisation of some silent SPNs was accompanied by action potential firing and of active SPNs by an increased frequency of action potential firing. The L-CCG-I induced depolarisation was dose-dependent and was maintained in the presence of TTX (500 nM, $n = 2$). In six SPNs L-CCG-I elicited dose-dependent hyperpolarisations (Fig. 2B). In one of these neurones the hyperpolarising response was converted to a depolarisation by increasing the duration of perfusion or the concentration of agonist. Responses to L-CCG-I and (1*S*,3*R*)-ACPD were compared in 17 SPNs. In 13 SPNs L-CCG-I and (1*S*,3*R*)-ACPD both elicited depolarising responses (Fig. 2A). In four SPNs (1*S*,3*R*)-ACPD caused a depolarisation and L-CCG-I caused a hyperpolarisation (Fig. 2B).

(*S*)-4C3HPG is an antagonist at group I receptors and an agonist at group II receptors (Watkins and Collingridge, 1994; Pin and Duvoisin, 1995). Hyperpolarising responses accompanied by a reduction in the frequency of action potential firing were observed from two of six SPNs in response to perfusion of (*S*)-4C3HPG (100–300μM; Fig. 2C). The remaining neurones showed no change in membrane potential, input resistance or spontaneous activity. Of the two SPNs hyperpolarised by (*S*)-4C3HPG, one depolarised and one hyperpolarised in response to (1*S*,3*R*)-ACPD. The four SPNs not responding to (*S*)-4C3HPG showed depolarising responses to (1*S*,3*R*)-ACPD. The (*S*)-4C3HPG-induced hyperpolarisation was maintained in the presence of 500 nM TTX (Fig. 2C). Perfusion of DCG-IV (2–5μM), a potent group II agonist (Pin and Duvoisin, 1995), hyperpolarised three of eight SPNs tested (data not shown). Hyperpolarisations induced by DCG-IV were observed in the presence of the NMDA receptor antagonist D-APV (20 μM), indicating that they did not involve activation of NMDA receptors.

L-AP4 is an agonist at group III mGluRs (Nakajima et al., 1993; Pin and Duvoisin, 1995; Saugstad et al., 1997). Perfusion of L-AP4 (100 μM; 30–120 s) onto five SPN, that depolarised in response to (1*S*,3*R*)-ACPD, had no effect on their membrane potential, input resistance or spontaneous activity (data not shown).

3.2. Effects of antagonists on mGluR-mediated depolarisation of SPNs

The actions of phenylglycine derived antagonists on the mGluR agonist-induced depolarisation of SPNs were examined. (+)MCPG has antagonist activity at group I and II mGluRs (Watkins and Collingridge, 1994; Pin and Duvoisin, 1995). In the presence of 200–500 μM (+)MCPG, depolarisations induced by (1*S*,3*R*)-ACPD ($n = 8/8$), DHPG ($n = 4/4$) and L-CCG-I ($n = 2/2$) were reversibly suppressed (Fig. 3). (+)MCPG alone had no direct effects on the membrane potential, input resistance or spontaneous activity. (*S*)-4CPG selectively antagonises group I mGluRs (Watkins and Collingridge, 1994; Pin and Duvoisin, 1995). In the presence of 500 μM (*S*)-4CPG depolarising responses induced by (1*S*,3*R*)-ACPD ($n = 5/5$), DHPG ($n = 3/3$) and L-CCG-I ($n = 4/4$) were reversibly suppressed (Fig. 4). Hyperpolarising responses were not unmasked by (*S*)-4CPG in any of the neurones tested. (*S*)-4CPG also has agonist activity at mGluR2, with an EC_{50} of 500 μM (Hayashi et al., 1994) and so might

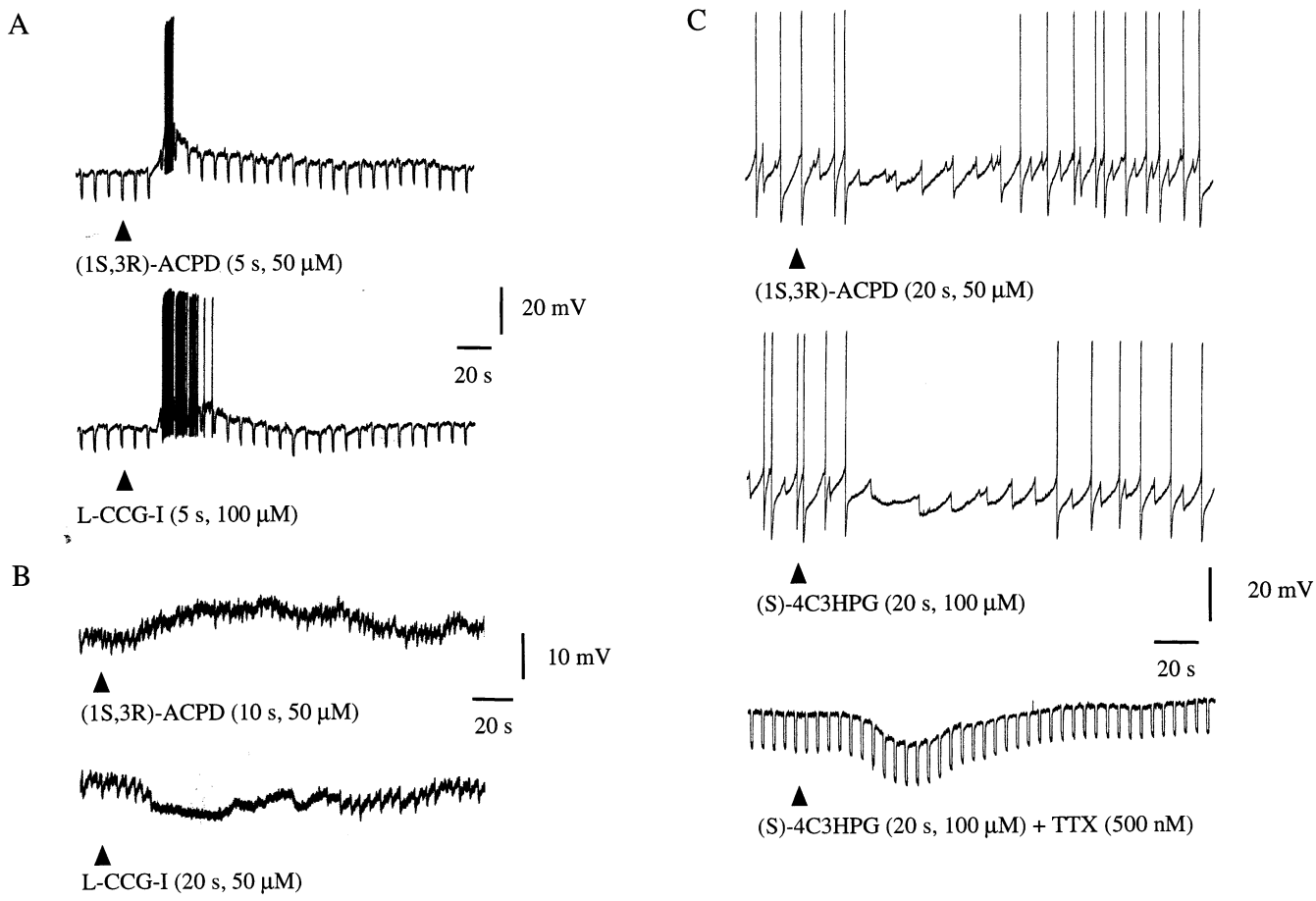

Fig. 2. Agonist pharmacology of mGluR mediated hyperpolarisation of SPNs. (A) Example of an SPN (Em −62 mV) which depolarised following perfusion of (1*S*,3*R*)-ACPD or L-CCG-I. (B) Example of an SPN (Em −60 mV) in which perfusion of (1*S*,3*R*)-ACPD caused a depolarisation and L-CCG-I an hyperpolarisation. (C) Perfusion of (1*S*,3*R*)-ACPD onto this SPN (Em −55 mV) caused a hyperpolarisation and reduction in the frequency and amplitude of spontaneous membrane potential oscillations. Perfusion onto the same SPN of (*S*)-4C3HPG also caused a hyperpolarisation and reduction in the frequency and amplitude of spontaneous membrane potential oscillations. The (*S*)-4C3HPG induced response was maintained in 500 nM TTX indicating that it involved a direct action on SPNs.

have been expected to have agonist effects. It showed no apparent agonist effects on membrane potential, input resistance or spontaneous activity of five SPNs and appeared to cause a small hyperpolarisation (<2 mV) in one SPN. Recovery of agonist responses from the effects of antagonists usually occurred within 20 min of switching the perfusion medium back to control aCSF.

3.3. Induction and modulation of membrane potential oscillations by mGluR agonists

Subthreshold oscillations in the membrane potential of SPNs occur due to conduction of action potentials from adjoining electrotonically-coupled SPNs and may be important for the generation of rhythmic patterns of sympathetic activity (Logan et al., 1996). In this study SPNs were divided into two groups on the basis of their spontaneous activity in the absence of any drugs: those demonstrating spontaneous subthreshold membrane potential oscillations were termed spontaneously oscillating SPNs; those that did not demonstrate spontaneous subthreshold oscillations (equivalent to silent and active SPNs described in Logan et al., 1996) were termed non-oscillating SPNs. mGluR agonists could induce oscillations in the membrane potential of non-oscillating SPNs and had variable actions on the pattern of spontaneously occurring oscillations. Examples from three different SPNs of the effects of (1*S*,3*R*)-ACPD on the pattern of subthreshold activity are shown in Fig. 5.

Biphasic membrane potential oscillations of similar waveform to those seen to occur spontaneously were induced by (1*S*,3*R*)-ACPD in 31 of 95 non-oscillating SPNs (33%; Fig. 5A). The oscillations were seen to accompany depolarising responses and were of a similar duration. Oscillations were induced in a similar proportion of non-oscillating SPNs in response to DHPG (10/36, 28%), but in a smaller proportion in response to L-CCG-I (2/17, 11%). Subthreshold membrane poten-

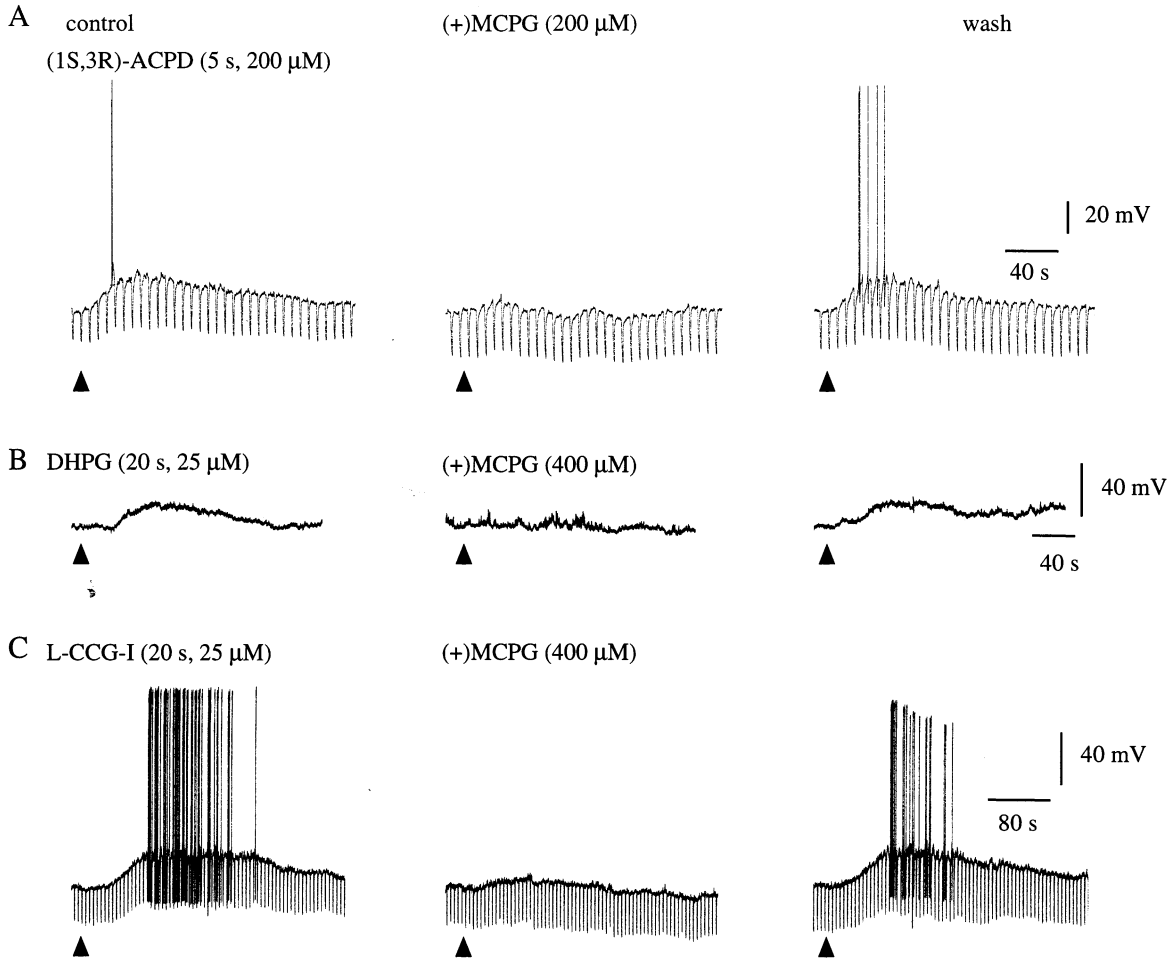

Fig. 3. (+)MCPG, an antagonist of group I and group II mGluRs, reversibly depressed mGluR agonist induced depolarisation of SPNs. Shown are the effects of (+)MCPG on responses of three different SPNs to mGluR agonists (1*S*,3*R*)-ACPD (A), DHPG (B) and L-CCG-I (C). Estimated membrane potentials of the neurones were −70 mV (A), −60 mV (B) and −55 mV (C).

tial oscillations were not induced by compounds lacking agonist activity at group I mGluRs.

mGluR agonists had variable effects on the pattern of spontaneous membrane potential oscillations. Perfusion of (1*S*,3*R*)-ACPD increased the frequency of oscillations in nine neurones (Fig. 5C) and reduced the frequency in four neurones (Fig. 5B). Perfusion of DHPG increased the frequency of oscillations in all five SPNs tested, whereas L-CCG-I increased the frequency in one SPN and reduced the frequency in two SPNs. Both of the cells responding to (*S*)-4C3HPG showed spontaneous membrane potential oscillations that were reduced in frequency during the response (Fig. 2C). Agonist-induced increases in the frequency of oscillations were always accompanied by depolarising responses, whereas reductions in frequency coincided with either hyperpolarisations or no change in membrane potential.

4. Discussion

In this study we have shown that mGluR agonists can have direct excitatory and inhibitory actions on the membrane properties of SPNs. The pharmacological profile of the mGluR mediated excitation resembles that of group I mGluRs implying that the response is mediated by either mGluR1 or mGluR5. Activation of group I mGluRs could also induce or increase the frequency of subthreshold membrane potential oscillations. An mGluR agonist-induced hyperpolarisation and inhibition of membrane potential oscillations was observed in a minority of SPNs and was mediated by a receptor with an agonist profile resembling that of group II mGluRs. These results suggest that glutamatergic synaptic inputs to SPNs may have inhibitory as well as excitatory actions.

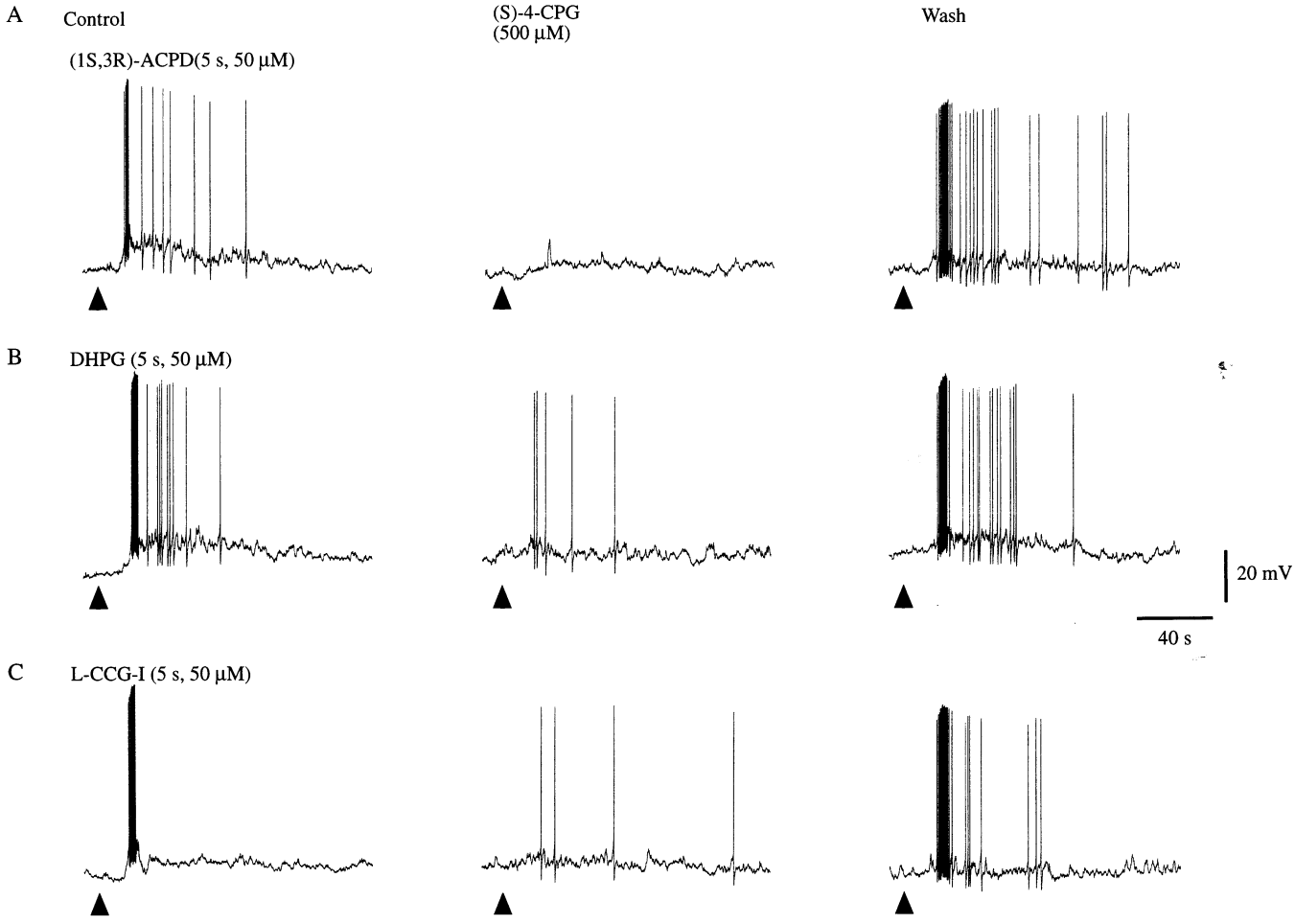

Fig. 4. (*S*)-4CPG (500 μM), a selective group I mGluR antagonist, reversibly depressed depolarising responses to (1*S*,3*R*)-ACPD, DHPG and L-CCG-I. All responses were recorded from the same neurone (Em −55 mV).

4.1. mGluR agonist-induced depolarisations

The mGluR agonists that depolarised SPNs (1*S*,3*R*)-ACPD, L-quisqualate, CHPG, DHPG and L-CCG-I, all have agonist activity at mGluRs from group I (Watkins and Collingridge, 1994; Gereau and Conn, 1995; Pin and Duvoisin, 1995; Doherty et al., 1997). The depolarisations elicited by these agonists were maintained in TTX indicating that the agonists were acting at postsynaptic receptors on SPNs. DHPG has been reported to be a selective agonist at group I mGluRs (Gereau and Conn, 1995), however it also has some agonist activity at mGluR6 (Sekiyama et al., 1996). The response to DHPG was unlikely to have been mediated by mGluR6 as L-AP4, which activates mGluR6 with an EC_{50} of 0.9 μM (Nakajima et al., 1993), had no effect on the membrane potential or input resistance of SPNs at a concentration of 100 μM. Therefore, the actions of DHPG indicate that SPNs express group I mGluRs which when activated cause neuronal depolarisation.

Both (1*S*,3*R*)-ACPD and L-CCG-I have agonist activity at mGluRs from groups I, II and III (Pin and Duvoisin, 1995; Saugstad et al., 1997). Depolarisations evoked by these agonists may therefore not have involved activation of mGluRs from group I. To test for the involvement of group II receptors, agonists that are more selective for group II responses (1*S*,3*S*)-ACPD (Pook et al., 1992) and (*S*)-4C3HPG (Watkins and Collingridge, 1994), were applied to SPNs. At concentrations that would be sufficient to selectively activate group II mGluRs, neither compound depolarised SPNs indicating that activation of group II mGluRs does not depolarise SPNs. Similarly, responses were not observed to the selective group III agonist L-AP4 at concentrations over 100 times its EC_{50} at mGluR4, mGluR6 and mGluR8 (Pin and Duvoisin, 1995; Saugstad et al., 1997), suggesting that these receptors do not modulate the excitability of SPNs. In a previous study L-AP4 at a concentration of 0.1–1 mM, sufficient to activate mGluR7 (EC_{50} 160 μM), depolarised SPNs (Spanswick et al., 1995). This suggests that activation of mGluR7 may depolarise SPNs, however at such high concentrations non-specific effects of L-AP4 can not be ruled out. The depolarising responses observed in this

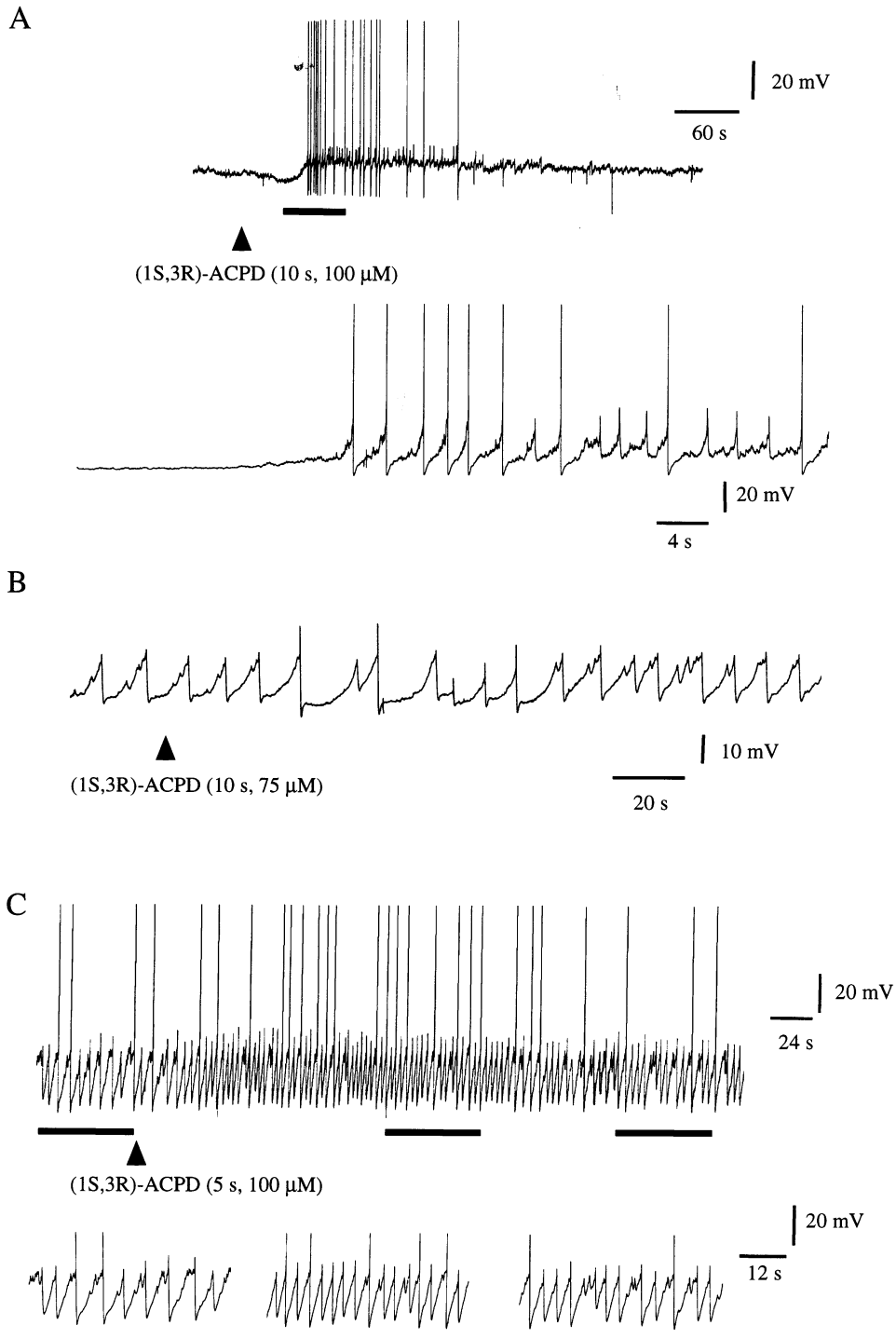

Fig. 5. Examples of induction and modulation of membrane potential oscillations by (1*S*,3*R*)-ACPD. The data shown are from three different SPNs. (A) Depolarisation of this silent SPN (Em −55 mV) by (1*S*,3*R*)-ACPD was accompanied by induction of membrane potential oscillations. Inset shows onset of the responses on an expanded time base, corresponds to period above the bar on the main trace. (B) Perfusion of (1*S*,3*R*)-ACPD onto this spontaneously oscillating SPN (Em −60 mV) decreased the frequency and increased the amplitude of the oscillations. (C) Perfusion of (1*S*,3*R*)-ACPD increases the frequency of spontaneous oscillations in the membrane potential of this SPN (Em −50 mV). Inset shows membrane potential on an expanded time base for the periods corresponding to bars beneath the main trace.

study were therefore unlikely to have been mediated by mGluRs from group II or group III.

Examination of the effects of phenylglycine antagonists on mGluR agonist induced depolarisations confirmed the involvement of a group I type receptor. (*S*)-4CPG, which selectively antagonises group I mGluRs (Pin and Duvoisin, 1995), reversibly depressed depolarisations induced by (1*S*,3*R*)-ACPD, L-CCG-I

and DHPG. The depolarising responses to these three agonists were also depressed by the broad spectrum mGluR antagonist (+)MCPG. Comparison of the effects of (+)MCPG and (*S*)-4CPG on mGluR1α and mGluR5a has suggested that both compounds may be relatively selective for mGluR1α (Brabet et al., 1995; Joly et al., 1995; Kingston et al., 1995). If the other splice variants of mGluR1 and mGluR5 show similar sensitivities to these phenylglycines, this would suggest that the mGluR mediated depolarisation of SPNs is mediated by either mGluR1 or a novel receptor with similar pharmacological characteristics. However, depolarising responses were evoked by CHPG which is reported to be inactive at mGluR1α, but active at mGluR5a (Doherty et al., 1997), indicating that depolarisations may be mediated by mGluR5. The precise identity of the mGluRs expressed by SPNs will remain unresolved until complementary data becomes available from immunohistochemical or *in situ* hybridisation studies.

Modulation of accommodation and spike frequency adaption by mGluRs has previously been observed in SPNs (Spanswick et al., 1995) and other neurones (Baskys, 1992), but was not consistently observed in this study. This could either be a result of using whole-cell patch-clamp rather than intracellular recording methods or carrying out the experiments at room temperature rather than 34°C as in the previous study. Pharmacological characterisation of similar mGluR mediated responses during intracellular recordings from hippocampal pyramidal neurones suggests they are mediated by group I receptors (Davies et al., 1995; Gereau and Conn, 1995). As described previously (Spanswick et al., 1995), consistent changes in SPN input resistance were not observed in this study during perfusion of mGluR agonists. This may reflect the involvement of more than one ionic conductance in the membrane polarisations, localisation of mGluRs to distal dendritic regions, or the effects of electrotonic coupling between SPNs.

4.2. mGluR agonist-induced hyperpolarisations

A sub-population of SPNs were hyperpolarised by agonists with activity at group II mGluRs. The hyperpolarisation was maintained in TTX indicating that it involved a direct effect of the agonists on SPNs. The hyperpolarisation can not be attributed to a reversal of the mGluR agonist induced depolarisation for a number of reasons: individual SPNs demonstrated either depolarisations or hyperpolarisations from the same initial membrane potential in response to perfusion of different agonists; the mGluR agonist-induced depolarisation is relatively insensitive to membrane potential over the ranges used in this study (Spanswick et al., 1995) and the mGluR agonist-induced depolarisation and hyperpolarisation had different agonist profiles. The proportion of neurones hyperpolarised by each agonist could be related to the relative selectivity of each agonist for group II over group I receptors. (*S*)-4C3HPG and DCG-IV which do not have agonist activity at group I receptors induced hyperpolarisations in the greatest proportion of neurones (2/6, 33% and 3/8, 37.5%, respectively), followed by L-CCG-I (5/24, 21%) and then (1*S*,3*R*)-ACPD (3/143, 2%). Hyperpolarisations were not observed in response to (1*S*,3*S*)-ACPD, possibly due to the small sample of neurones tested, or to the pharmacological properties of the receptor mediating the hyperpolarisation. DHPG, which is inactive at group II mGluRs, did not induce hyperpolarising responses in any of the neurones tested, including SPNs that hyperpolarised in response to other mGluR agonists. It is possible that depolarisation of SPNs by (1*S*,3*R*)-ACPD and L-CCG-I acting at group I mGluRs may have masked hyperpolarising effects of these agonists mediated by group II mGluRs. Even so, mGluRs mediating hyperpolarising responses appeared to be present in a minority of SPN, as perfusion of (1*S*,3*S*)-ACPD, (*S*)4C3HPG, (*S*)4CPG or DCG-IV at doses that would have been sufficient to activate group II, but not group I receptors, did not induce hyperpolarising responses in the majority of SPNs tested.

Hyperpolarising responses mediated by mGluRs also occur in the basolateral amygdala, where in response to mGluR agonists a large proportion of neurones either hyperpolarise or show biphasic responses, consisting of a hyperpolarisation followed by a depolarisation (Holmes et al., 1996). The hyperpolarisation of amygdala neurones is mediated by group II mGluRs and is associated with activation of Ca^{2+} activated K^+ channels (Holmes et al., 1996).

4.3. mGluR mediated changes in oscillatory activity

Biphasic oscillations in the membrane potential of SPNs are a consequence of electrotonic coupling between groups of SPNs (Logan et al., 1996). Hence, an action potential fired by a neurone is filtered as it is conducted through the electrotonic junctions resulting in a biphasic membrane potential oscillation in each adjoining neurone. These oscillations may underlie the generation of rhythmic and synchronous patterns of action potential firing by SPNs (Logan et al., 1996). The mGluR agonist-induced changes in the pattern of oscillatory activity may reflect modulation of the gap junctions mediating the coupling or changes in the membrane properties of the neurone driving the oscillatory activity. In the latter case induction or increase in the frequency of oscillations may be accounted for by depolarisations and increased action potential firing by the neurone driving the oscillations. Conversely a reduction in the frequency may be accounted for by

hyperpolarisation and reduction in the frequency of action potential firing by the driver neurone. In support of this explanation, a preliminary study suggests that oscillations are induced as a result of suprathreshold depolarisation of coupled silent SPNs (Nolan and Logan, 1994). Furthermore the agonist profile of the observed changes in the pattern of oscillations suggested that induction or increases in the frequency of oscillations, like the depolarisation, were mediated by a group I mGluR and decreases in the frequency of oscillations, like the hyperpolarisation, were mediated by a group II mGluR. It is also interesting to note that inhibitory responses to (1*S*,3*R*)-ACPD and L-CCG-I occurred in a high proportion of spontaneously oscillating SPNs compared to the total population of SPNs, indicating that a relatively high proportion of electrotonically-coupled SPNs may express group II mGluRs. Examination of the effects of mGluR agonists during simultaneous recordings from pairs of SPNs will be necessary in order to determine the mechanism by which oscillations are induced.

4.4. Functional significance of mGluRs expressed by SPNs

The functional role of mGluRs expressed by SPNs in relation to the generation of sympathetic output remains to be determined. The results described here suggest that group I mGluRs expressed by most or all SPNs may mediate increases in sympathetic output and group II mGluRs expressed by a sub-population of SPNs may mediate decreases in sympathetic output. During *in vivo* experiments, microinjection of ACPD into the spinal thoracic intermediolateral cell column, the region containing the majority of SPN cell bodies, had no effect on heart rate or blood pressure (Arnolda et al., 1996). The lack of response to ACPD in this study could be accounted for if the excitatory actions of ACPD were masked by inhibitory effects directly on SPNs or on the excitatory input to SPNs (Wu and Dun, 1993). The use of compounds with more selectivity between mGluR subtypes to examine the role of mGluRs expressed by SPNs *in vivo* may help address this issue. A further requirement for understanding the physiological role of mGluRs is to isolate synaptic responses mediated by these receptors. In other areas of the central nervous system mGluR mediated excitatory postsynaptic responses have rise times and durations considerably longer than those mediated by iGluRs (Glaum and Miller, 1992; Batchelor et al., 1994; Pozzo Miller et al., 1995). If similar synaptic inputs onto SPNs occur, then mGluRs may be expected to mediate relatively prolonged EPSPs compared to the fast EPSPs mediated by iGluRs (Inokuchi et al., 1992; Deuchars et al., 1995). Although central, synaptically-evoked mGluR-mediated IPSPs have not been reported, such a response may serve to curtail depolarisations produced by prolonged periods of excitatory synaptic activity. The possibility that some glutamatergic inputs to SPNs may be capable of inhibitory as well as excitatory effects on sympathetic output could have important implications for our understanding of how SPNs contribute to the generation of an integrated pattern of sympathetic output.

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