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Differential actions of spinal analgesics on mono-versus polysynaptic Aδ-fibre-evoked field potentials in superficial spinal dorsal horn in vitro

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Abstract

Processing of nociceptive information can be modulated at various levels in spinal cord that may range from changes of neurotransmitter release from primary afferent Aδ- or C-fibres to excitability changes of spinal interneurones or motoneurones. The site and mechanism of action of spinal analgesics has been assessed with a number of in vivo and in vitro methods with sometimes conflicting results. Here, we have used transverse spinal cord slices with attached dorsal roots to simultaneously record mono- and polysynaptic Aδ-fibre-evoked field potentials in superficial spinal dorsal horn. Two classical spinal analgesics, morphine and clonidine, and the metabotropic glutamate receptor agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD) differentially affected mono- and polysynaptic Aδ-fibre-evoked transmission in spinal dorsal horn. Polysynaptic responses were dose-dependently inhibited while the monosynaptic response remained unaffected. These results suggest that spinal analgesics may preferentially affect polysynaptic but not monosynaptic Aδ-fibre-evoked responses in superficial spinal dorsal horn. © 2000 International Association for the Study of Pain. Published by Elsevier Science B.V. All rights reserved.

Keywords: μ-Opiate receptor; α_2-Adrenoceptor; Metabotropic glutamate receptor; Rat; Antinociception

1. Introduction

Nociceptive primary afferent Aδ- and C-fibres terminate in superficial spinal dorsal horn where they make exclusively excitatory synaptic contacts with second order neurones. Nociceptive information is then transmitted via intercalated interneurones to ascending tract neurones and to motoneurones. Processing of nociceptive information can be inhibited or facilitated at all levels within spinal dorsal and ventral horns. Powerful endogenous antinociceptive systems exist that depress spinal nociception via activation of opioid receptors (mainly μ- and δ-opioid receptors), monoaminergic receptors (5-HT receptors and α_2-adrenoceptors) and amino acid receptors ($GABA_{A/B}$ and glycine receptors). Spinal application of selective agonists at μ-opioid-receptors or α_2-adrenoceptors such as morphine and clonidine is now being used to treat acute and chronic pain (Onofrio and Yaksh, 1990; Eisenach et al., 1996). Intrathecal injections in awake, otherwise drug-free animals or humans (Yaksh, 1981; Eisenach et al., 2000) resemble the clinical situation but may be subject to potentially confounding effects from attention, changes of blood pressure, sedation and/or motor impairment. A number of in vitro methods have been developed such as recording of dorsal root-evoked ventral root potentials (Faber et al., 1997). These motor responses are subject to drug effects in the motor system or ventral horn. For example μ-opiate receptors are also present in spinal cord ventral horn (Gouarderes et al., 1991; Ding et al., 1996). It is important to identify the afferent fibre type that evokes the response in spinal cord because noxious mechanical and heat-evoked (Wegert et al., 1997), as well as Aδ- and C-fibre-evoked activities (Le Bars et al., 1976) may be differentially affected by spinal analgesics in vivo. Thus, spinal cord slice preparations should have long dorsal roots attached to allow identification of afferent fibre types by calculation of conduction velocity with sufficient precision. Evidence exists that the action of spinal analgesics may also be strikingly different at the first central synapse as compared to polysynaptic responses (Sastry and Goh, 1983; Magnuson and Dickenson, 1991).

Here, we have used a transverse spinal cord slice preparation with long dorsal roots attached to simultaneously record mono- and polysynaptic Aδ-fibre-evoked extracellular field potentials in the superficial spinal dorsal horn. The effects of morphine and clonidine, two clinically used spinal analgesics

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and an experimental neuroactive drug, the metabotropic glutamate receptor agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD) on synaptic transmission were assessed. Some of these results have been published in form of an abstract (Ruscheweyh and Sandkühler, 1999).

2. Methods

2.1. Preparation of spinal cord slices

Transverse, 500-μm thick slices each with one 7–14 mm long dorsal root attached were obtained from lumbar spinal cord of Sprague–Dawley rats (18–28 days old). Slices were incubated in a solution that consisted of (in mM): NaCl 124, KCl 5, KH_2PO_4 1.3, $CaCl_2$ 2.4, $MgSO_4$ 1.3, $NaHCO_3$ 26, Glucose 15 and that was equilibrated with 95% O_2, 5% CO_2, pH 7.4. A single slice was then transferred to the recording chamber where it was superfused with oxygenated recording solution at 3 ml/min. The recording solution was identical to the incubation solution except for (in mM) NaCl 127, KCl 1.9 and $CaCl_2$ 4.3. Experiments were conducted at room temperature (20–24°C).

2.2. Recording and stimulation techniques

Field potential recordings were made with glass microelectrodes from the superficial spinal dorsal horn. When filled with (in mM) NaCl 135, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5 (pH adjusted to 7.2 with NaOH), electrodes had DC tip resistances between 2 and 7 MΩ. A suction electrode was used for electrical stimulation of the dorsal root with a constant current stimulator (World Precision Instruments, Sarasota). Low-pass filter was set to 1000 Hz, amplification 500× (Axopatch 200B, Axon Instruments). The field potentials were digitized by an A/D converter card (Data Translation DT 2821) and stored in a computer for off-line analysis using the software package Experimenter's Workbench, version 4.0 (Data Wave Technologies).

2.3. Experimental protocol

Recording sites were optimized for two clearly distinguishable signals in response to dorsal root stimulation. Thresholds were around 0.1 mA (0.1 ms pulse width), latencies were approximately 2.5 ms for the first signal and 10–11 ms for the late signal, and amplitudes were at least 400 μV. Test pulses were given at 15-s intervals and at a supramaximal intensity (0.7 mA at 0.1 ms). In some experiments, stimulus–response curves were determined and/or responses to ten consecutive pulses at 1 and 10 Hz were recorded. After obtaining stable amplitudes for both signals for at least 15 min, a drug was added to the superfusion solution. One experiment was performed per slice.

2.4. Data analysis

Two consecutive field potentials were averaged and synaptic strength was quantified by measuring the baseline-to-peak amplitude of the first signal and the onset-to-peak amplitude of the second signal (Fig. 1A). The latencies from the beginning of the stimulus artefact to the peak of each signal were also measured. The mean amplitudes and the mean latencies of ten averaged test responses prior to the drug application served as controls. The mean amplitudes and latencies of ten consecutive averaged responses at the end of the drug application were used to assess the effect of the drug. For calculating the conduction velocity, the latency from the beginning of the artefact to the onset of the first signal was measured. For the analysis of the responses to 1- and 10-Hz stimulation the original not averaged field potentials were used. All values were expressed as mean ± SEM. Statistical comparisons were made using the nonparametric Wilcoxon rank sum test. $P < 0.05$ was considered significant. The software package Mathematica, version 3.0 (Wolfram Research Inc., Champaign, IL) was used.

2.5. Application of drugs

All drugs were added to the superfusion solution at known concentrations. Drugs and their sources were as follows: morphine hydrochloride (morphine; 1–3 μM; Merck), clonidine hydrochloride (clonidine, 30–1000 nM, Sigma), naloxone hydrochloride (naloxone, 10 μM, Sigma), (−)-bicuculline methiodide (bicuculline, 5 μM, Sigma), strychnine (2 μM, Sigma), D-2-amino-5-phosphovaleric acid (D-AP5, 25 μM, RBI), 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX, 5 μM, Tocris), (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD, 40 μM, Tocris), *S*-α-methyl-4-carboxyphenylglycine (*S*-MCPG, 1 mM, Tocris). Atipamezole hydrochloride (atipamezole, 1 μM) was kindly donated by Farmos Orion, Turku, Finland.

Stock solutions were prepared of each drug by dissolving morphine, clonidine, naloxone, atipamezole, D-AP5 and bicuculline in distilled water, strychnine and CNQX in dimethyl sulfoxide (DMSO, Sigma) and (1*S*,3*R*)-ACPD and *S*-MCPG in NaOH (10 mM and 50 nM, respectively). Stock solutions were stored in aliquots at −20°C.

3. Results

Extracellular field potentials were recorded in superficial spinal dorsal horn in response to electrical stimulation of the dorsal root (0.7 mA, 0.1 ms) in the presence of a high Ca^{2+} (4.3 mM) recording solution (Fig. 1A). An early and a late potential could be distinguished. The early potential had a latency of 2.50 ± 0.02 ms ($n = 7$) that corresponds to a calculated conduction velocity of 4.9 ± 0.5 m/s when synaptic delay is assumed to be 1 ms. The late potential appeared 7.8 ± 0.1 ms after the early potential ($n = 7$). Both potentials had almost identical stimulus–response functions (Fig. 1B), suggesting that they were generated by the same type of afferent nerve fibres. The thresholds

were low (0.05–0.1 mA at 0.1 ms) and the intensity of test stimulation used in all experiments (0.7 mA, 0.1 ms) was supramaximal for both potentials.

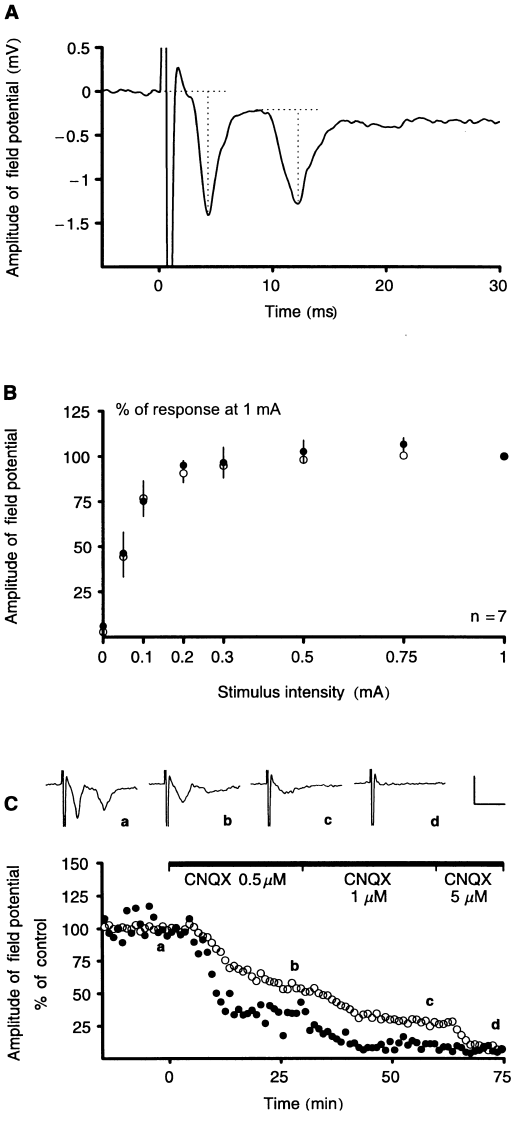

Fig. 1. Properties of Aδ-fibre-evoked field potentials in superficial spinal dorsal horn. (A) Illustration of original field potentials, evoked by electrical stimulation of the dorsal root (0.7 mA, 0.1 ms) and recorded in the superficial spinal dorsal horn. An early and a late potential following the artefact at time zero could easily be distinguished. The early potential had a latency of 2.5 ms that corresponds to a calculated conduction velocity of 4.9 ms. The late potential had a latency of approximately 10.3 ms. Amplitudes were measured from baseline to peak for the early potential and from onset to peak for the late potential as indicated by the dashed lines. (B) Mean stimulus-response functions for the early (open circles) and late potential (closed circles) at 0.1 ms pulse width were virtually identical in seven different slices. (C) Both potentials were abolished by bath application of the AMPA/kainate receptor antagonist CNQX (5 μM). CNQX inhibited the late potential more potently than the early potential. Original field potentials recorded immediately before application of CNQX (trace a) and after application of various concentrations of CNQX (traces b–d) are shown above the graph (calibration bars: 400 μV, 10 ms). The vertical bar indicates periods of superfusion at different concentrations of CNQX.

3.1. Effects of receptor antagonists of main excitatory and inhibitory neurotransmitters

Bath application of the NMDA receptor antagonist D-AP5 (50 μM) induced a small inhibition of the late (to 92 ± 3% of control after 30 min, $n = 7$, $P < 0.05$, data not shown) but not the early (101 ± 3% of control) potential. Both potentials were almost completely depressed (early potential to 9 ± 1% of control, late potential to 4 ± 1% of control, $n = 4$, $P < 0.01$) by bath application of the AMPA/kainate receptor antagonist CNQX (5 μM) in the presence of D-AP5 (50 μM). This indicates that both potentials require activation of postsynaptic ionotropic glutamate receptors of the AMPA/kainate subtype. The late potential was more rapidly and more deeply depressed by CNQX than the early potential (Fig. 1C).

Bath application of the $GABA_A$-receptor antagonist bicu-

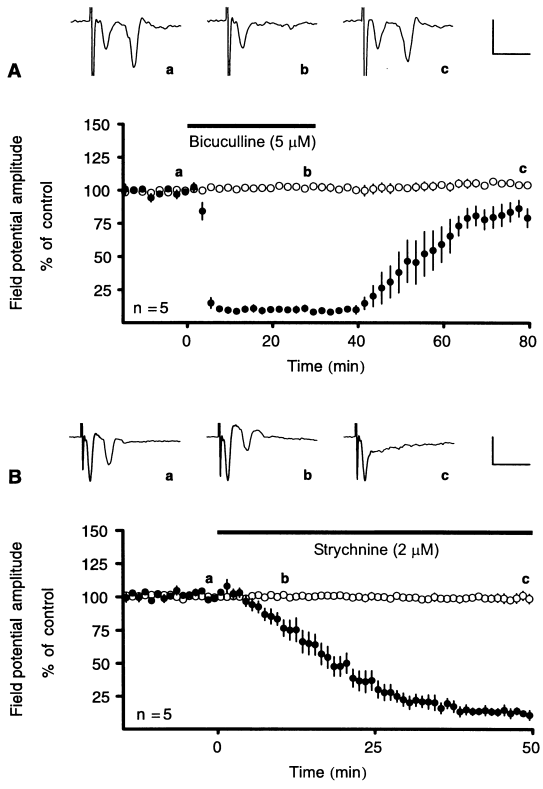

Fig. 2. Receptor antagonists of inhibitory neurotransmitters differentially affected early and late potentials. (A) Bath application of the $GABA_A$ receptor antagonist bicuculline (5 μM horizontal bar) induced a fast and complete depression of the late potential (closed circles) that was partially reversible at wash-out. The early potential (open circles) remained unaffected. Above the graph, original traces of field potentials recorded immediately before application of bicuculline (trace a), after wash-in (trace b) and after wash-out of bicuculline (trace c) are shown. (B) The glycine receptor antagonist strychnine (2 μM) induced a depression of the late potential that was slower but similar in magnitude (closed circles). The early potential (open circles) was not affected. Above the graph, original traces of field potentials recorded immediately before (trace a) and 12 and 50 min after application of strychnine (traces b,c) are shown (calibration bars in A and B: 500 μV, 10 ms).

culline (5 μM) induced a fast and reversible inhibition of the late (10 ± 2% of control after 30 min, $n = 5$, $P < 0.01$) but not the early potential (102 ± 1% of control, Fig. 2A). Application of the glycine receptor antagonist strychnine (2 μM) produced a slow depression of the late (13 ± 2% of control after 50 min, $n = 5$, $P < 0.01$) but not the early potential (99 ± 3% of control, Fig. 2B).

3.2. Early and late potentials are generated in the same region of the superficial spinal dorsal horn

In two experiments, detailed mappings of the field potential were obtained at 26 different recording sites throughout the spinal grey and white matter. An isopotential map was calculated for each moment in time after the stimulation using a polynomial fit. Fig. 3A shows the situation 3.5 ms after stimulation, corresponding to the latency to peak for the early potential. Fig. 3B shows the isopotential map 11.8 ms after stimulation, corresponding to the latency to peak for the late potential. The locations of the isopotential curves were similar for both potentials indicating that the two potentials were generated in the same region of the spinal cord.

3.3. Mono- versus polysynaptic nature of Aδ-fibre-evoked spinal field potentials

The early potential has properties of a monosynaptic Aδ-fibre-evoked potential with respect to calculated conduction velocity and stimulation threshold. The late potential has properties of a polysynaptic potential: The late potential had a coefficient of variation of the amplitude 2.7 times larger than the early potential (Fig. 4A, coefficient of variation of the amplitude of 30 consecutive field potentials: 0.068 ± 0.004 for the late potential, 0.025 ± 0.002 for the early potential, $n = 36$, $P < 0.01$ for the difference) and a jitter of response latencies that was 3.5 times larger than for the early potential (Fig. 4B, standard deviation of latencies of 30 consecutive field potentials: 0.28 ± 0.02 ms for the late potential, 0.08 ± 0.02 ms for the early potential, $n = 36$, $P < 0.01$ for the difference). When applying ten pulses at 10-Hz stimulation to the dorsal root, the late potential was more strongly depressed (to 12 ± 5% of control) than the early potential (to 44 ± 9% of control, $n = 10$, $P < 0.01$, Fig. 4E). In contrast, stimulation with ten pulses given at 1 Hz equally depressed the two potentials (to 72 ± 8% of control for the early potential and to 72 ± 15% of control for the late potential) in nine experiments (Fig. 4D). Lowering the Ca^{2+} concentration from 4.3 to 2.4 mM depressed the late potential (to 44 ± 8% of control after 30 min, $n = 7$, $P < 0.01$) but not the early potential (105 ± 2% of control, Fig. 4C).

3.4. Multiple spinal field potentials can be evoked by stimulation of dorsal root

The second ('late') potential consistently appeared about 8 ms after the early potential. In 38% of the experiments a small third potential and in one experiment a fourth potential were recorded with almost the same peak-to-peak latency of 8 ms (Fig. 5). This could be explained by a recurrent excitation in spinal dorsal horn.

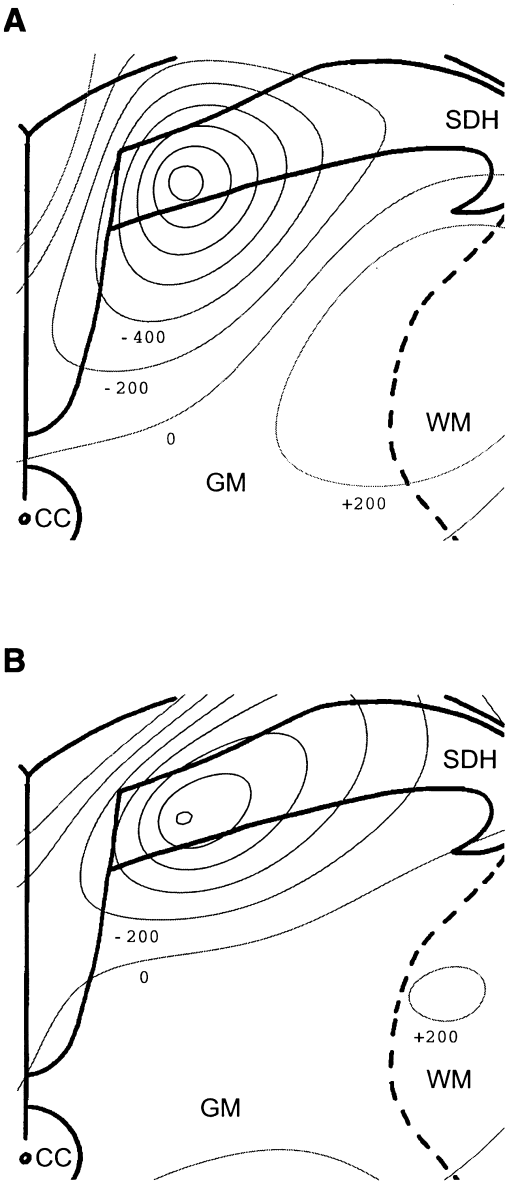

Fig. 3. Isopotential curves of Aδ-fibre-evoked field potentials of a representative experiment show that both potentials had their maximum amplitudes in the medial part of the superficial spinal dorsal horn. Isopotential curves were calculated on the base of recordings made at 26 different sites distributed throughout the white and grey matter of the slice with a focus on the ipsilateral dorsal horn. They are separated by 200 μV. A drawing of the corresponding dorsal horn section is superimposed. SDH, superficial dorsal horn; CC, central canal; GM, grey matter; WM, white matter. (A) Map of isopotential curves of the field potential measured at the peak latency of the early potential in this experiment (3.5 ms). (B) Map of isopotential curves of the field potential measured at the peak latency of the late potential in this experiment (11.8 ms).

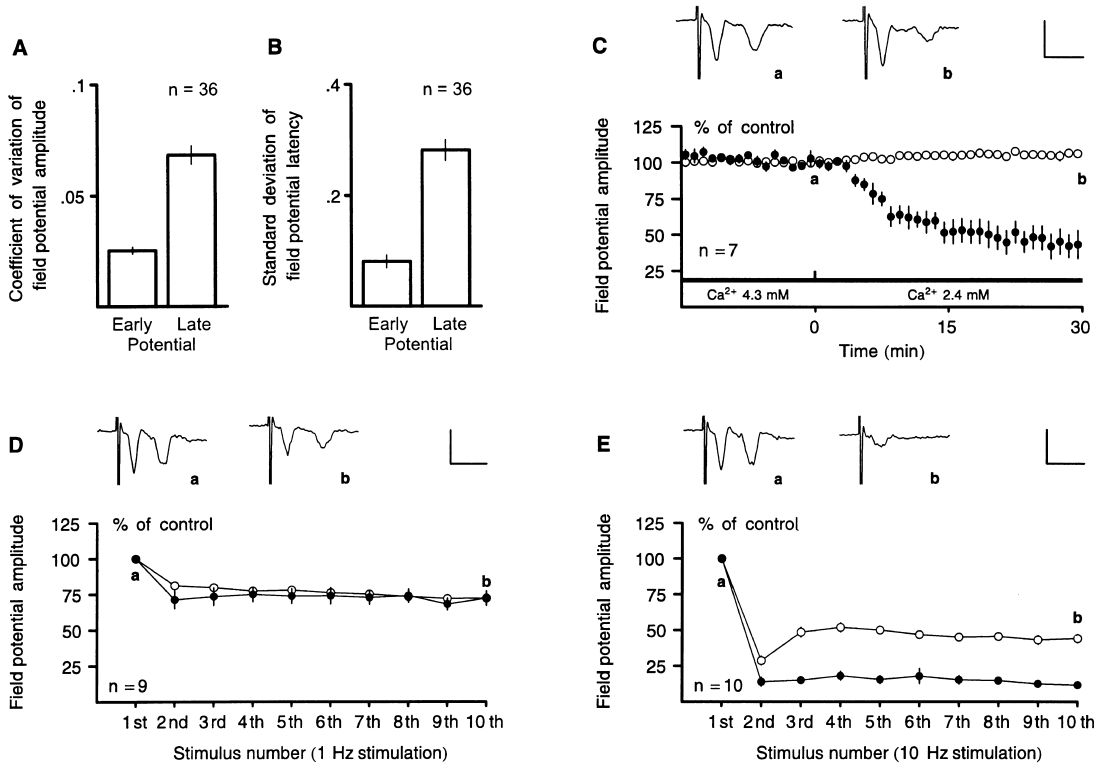

Fig. 4. Mono- vs. polysynaptic nature of the Aδ-fibre- evoked field potentials. (A) The mean coefficient of variation of the field potential amplitude of 30 consecutive field potentials as a measure of the variability of the field potential amplitude was significantly larger for the late potential than for the early potential in 36 experiments. (B) The mean standard deviation of the field potential latency of 30 consecutive field potentials as a measure of the field potential latency variability was significantly larger for the late potential than for the early potential in 36 experiments. (C) All experiments in this study were conducted in the presence of high Ca^{2+} (4.3 mM) to enhance the amplitude of the late potential. Lowering the Ca^{2+} concentration to 2.4 mM significantly depressed the late potential (closed circles) but did not affect the early potential (open circles). Above the graph, original traces of field potentials recorded immediately before lowering the Ca^{2+} concentration (trace a), and 30 min after lowering the Ca^{2+} concentration (trace b) are shown. (D,E) Ten pulses of 1 Hz stimulation depressed the early and the late potential to an equal extent. In contrast, stimulation with a higher frequency (ten pulses at 10 Hz) depressed the late potential more strongly than the early potential. Above the graphs, original traces of field potentials evoked by the first (trace a) and the tenth (trace b) pulse of 1- or 10-Hz stimulation, respectively, are shown (calibration bars in C–E: 500 μV, 10 ms).

3.5. Differential effects of spinal analgesics on the early and the late spinal field potential

The effects of morphine and clonidine on Aδ-fibre-evoked early and late field potentials were tested. When added to the recording solution, morphine (1–10 μM) and clonidine (30–1000 nM) dose-dependently inhibited the late potential without affecting the early potential. The depressions by morphine (1 μM) and clonidine (300 nM) were blocked by the opiate-receptor antagonist naloxone (10 μM) or the α_2-adrenoceptor antagonist atipamezole (1 μM), respectively. Naloxone (10 μM) and atipamezole (1 μM) alone did not significantly affect neither potential. Table 1 summarizes the effects and Figs. 6 and 7 show the time courses.

3.6. Differential effects of (1S,3R)-ACPD on early versus late spinal field potentials

When the metabotropic glutamate receptor agonist (1*S*,3*R*)-ACPD (40 μM) was added to the bath solution a fast and strong depression of the late potential was induced (to 36 ± 4% of control after 20 min, $n = 4$, $P < 0.01$). The depression by (1*S*,3*R*)-ACPD was fully reversible (100 ± 2% of control for the early potential, 101 ± 5% of control for the late potential 25 min after wash-out). The inhibition of the late potential by (1*S*,3*R*)-ACPD was partially blocked by the non-specific metabotropic glutamate receptor antagonist *S*-MCPG (1 mM, depression to 79 ± 6% of control at 20 min, $n = 2$). The early potential was not affected by (1*S*,3*R*)-ACPD (96 ± 1% of control, Fig. 8).

3.7. A positive correlation exists between amplitude reduction and increase in latency of the late spinal field potential

The reduction of amplitude of the late potential by either drug was accompanied by an increase in latency. This correlation is illustrated in Fig. 9A (coefficient of correlation: 0.59). Fig. 9B shows an example.

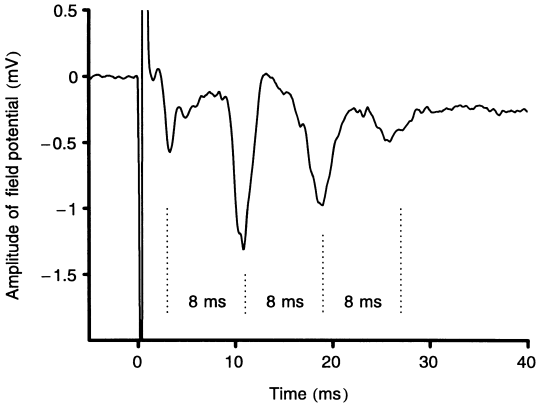

Fig. 5. In one experiment, four potentials were generated in the superficial spinal dorsal horn in response to electrical stimulation of the dorsal root. Peak-to-peak intervals were approximately 8 ms for all peaks. One possible explanation for this phenomenon would be the existence of a recurrent excitation in spinal cord dorsal horn.

4. Discussion

In spinal dorsal horn transmission of nociceptive information can be modulated at all levels, ranging from presynaptic modulation of neurotransmitter release at the central terminals of primary afferent Aδ- or C fibres to pre- and postsynaptic modulation at higher order neurones. It is desirable to differentially monitor modulation at the first synapse versus modulation in polysynaptic pathways of Aδ- and C-fibre-evoked activities. For example, the antinociceptive potency of spinal analgesics may vary considerably at different levels of the neuraxis (Sastry and Goh, 1983; Magnuson and Dickenson, 1991). In addition, central sensitization may be due to changes in neurotransmission at different levels of nociception (Lin et al., 1996; Sandkühler, 1996; Woolf, 1996). Here, we have characterized an in vitro method to simultaneously monitor mono- and polysynaptic field potentials that were evoked by impulses in primary afferent Aδ-fibres. We found that two clinically used spinal analgesics, morphine and clonidine, and an experimental drug, the metabotropic glutamate receptor agonist (1*S*,3*R*)-ACPD, preferentially affected polysynaptic but not monosynaptic Aδ-fibre-evoked responses in spinal dorsal horn. We propose that this in vitro method may be suitable to evaluate the effects of neuroactive compounds at defined steps of nociception in spinal dorsal horn.

4.1. Characteristics of Aδ-fibre-evoked field potentials recorded in superficial spinal dorsal horn

The mean latency of the late potential was consistently about 8 ms longer than that of the early potential. However, both potentials were elicited by impulses in the same type of primary afferent fibres as indicated by virtually identical stimulation thresholds and stimulus-response functions. It is likely that the late field potential involves three to four intercalated neurones that form a polysynaptic pathway if delay to spike initiation is considered to be 1.5–2 ms (Berry and Pentreath, 1976). In line with this, the late field potential was depressed more strongly by 10-Hz stimulation and displayed a larger jitter in latency and higher variability in amplitude than the early potential (Berry and Pentreath, 1976; Yoshimura and Jessell, 1989). Moreover, the late potential was significantly enhanced when the extracellular Ca^{2+} concentration was raised while the early potential was not affected.

Mapping studies revealed that both field potentials were generated at similar sites in superficial spinal dorsal horn. This could indicate that the intercalated neurones formed a reverberant circuit. Whenever a third or forth field potential was recorded they appeared at equal intervals of approximately 8 ms. This is consistent with the hypothesis of a recurrent excitation. The cytoarchitecture of spinal dorsal horn provides ample possibilities for reverberant pathways (Gobel, 1978; Woolf and King, 1987; Light and Kavookjian, 1988) possibly leading to rhythmic discharges of nociceptive spinal dorsal horn neurons (Eblen-Zajjur and

Table 1
Effects of morphine and clonidine on Aδ-fibre-evoked early and late field potentials[a]

	Concentration	*n*	Early potential		Late potential	
Morphine	1 μM	6	105 ± 3		86 ± 4	*
	3 μM	5	97 ± 2	n.s.	71 ± 7	**
	10 μM	5	95 ± 1	**	73 ± 7	**
Naloxone	10 μM	3	102 ± 4	n.s.	99 ± 1	n.s.
Naloxone/morphine	10 μM/3 μM	3	102 ± 0		96 ± 2	†
Clonidine	30 nM	5	104 ± 2	n.s.	90 ± 4	n.s.
	100 nM	6	98 ± 2	n.s.	72 ± 5	**
	300 nM	5	96 ± 4	n.s.	70 ± 6	**
	1 μM	5	108 ± 3	n.s.	68 ± 7	n.s
Atipamezole	1 μM	3	101 ± 3	n.s.	104 ± 8	n.s.
Atipamezole/clonidine	1 μM/300 nM	3	101 ± 3	n.s.	99 ± 8	n.s.

[a] *n*, number of experiments; n.s., not significantly different from control ($P > 0.05$);*significantly different from control ($P < 0.05$); **significantly different from control ($P < 0.01$); †significantly different from morphine (3 μM) alone ($P < 0.01$), also significantly different from control ($P < 0.01$).

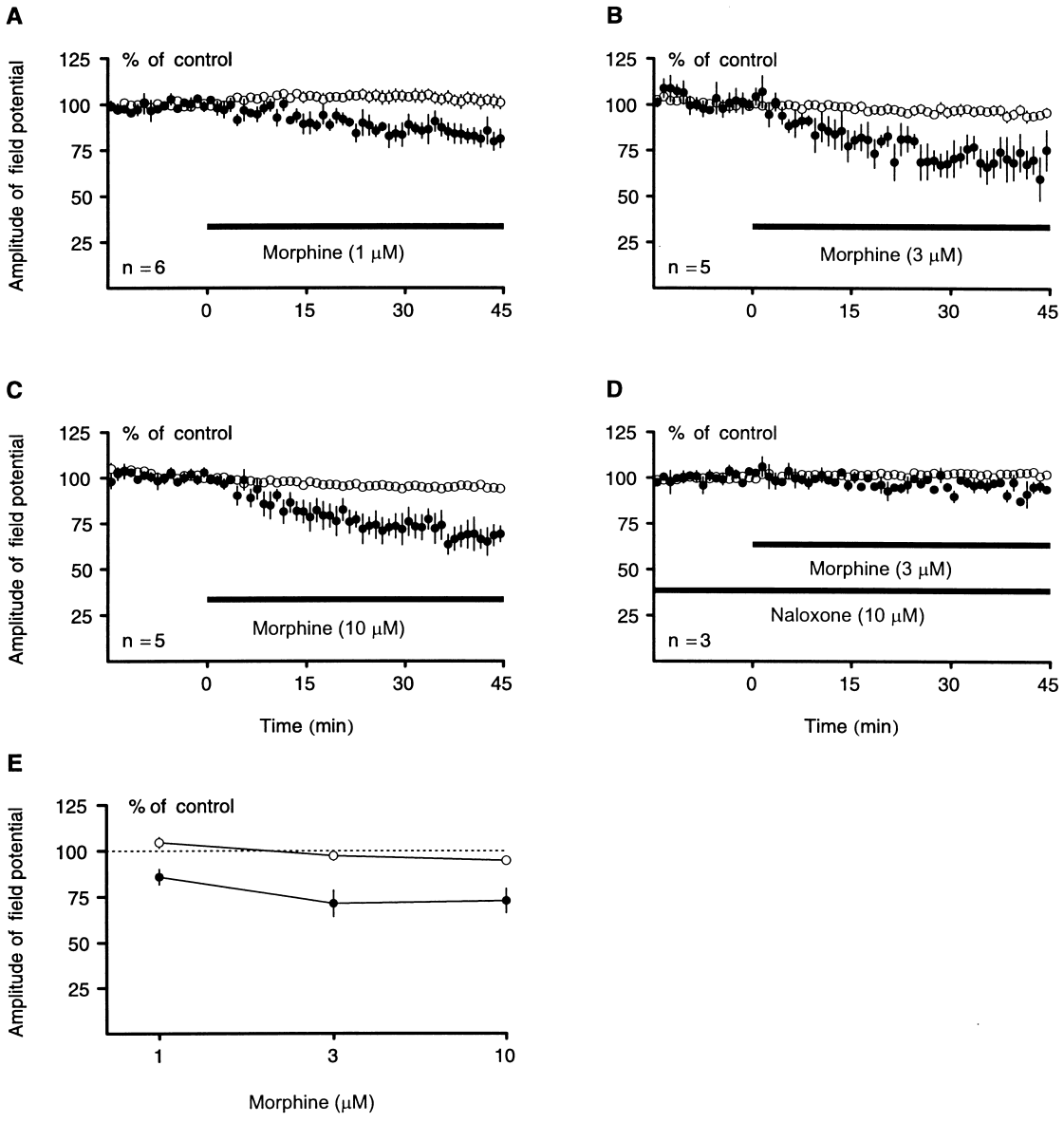

Fig. 6. Morphine dose-dependently depressed the late potential (closed circles) but not the early potential (open circles). (A–C) Mean time courses of inhibition by different concentrations of morphine (1–10 μM horizontal bars) are shown. (D) In the presence of naloxone (10 μM), morphine (3 μM) failed to depress the late potential. (E) The dose–response curves for morphine are shown.

Sandkühler, 1997). It has been proposed that a positive feedback mechanism may amplify the transmission of nociceptive information in spinal dorsal horn (Cadden, 1993; Sandkühler, 1996).

4.2. Neurotransmitter receptors involved in the generation of mono- and polysynaptic field potentials

It is generally accepted that glutamate or a related amino acid is the neurotransmitter of primary afferent nerve fibres, including Aδ-fibres (Gerber and Randic, 1989; Yoshimura and Jessell, 1990). Fast synaptic transmission is mediated by activation of AMPA/kainate-receptors on second order neurones (Yoshimura and Jessell, 1990). Activation of NMDA receptors typically adds a slow component to the monosynaptic EPSP, with little effect on amplitudes (Gerber and Randic, 1989; Yoshimura and Jessell, 1990). Consistently in our experiments, field potentials were abolished by bath application of the AMPA/kainate-receptor antagonist CNQX, while the NMDA-receptor antagonist D-AP5 had no effect on amplitudes of the monosynaptic field potentials. Polysynaptic responses may be more sensitive to NMDA-receptor blockade (Yoshimura and Jessell, 1990) and consistently, the late potential was slightly but significantly depressed by D-AP5.

Stimulation of primary afferent Aδ-fibres may also excite glycinergic or GABAergic inhibitory neurones in spinal dorsal horn. Aδ-fibre-evoked disynaptic IPSPs share some

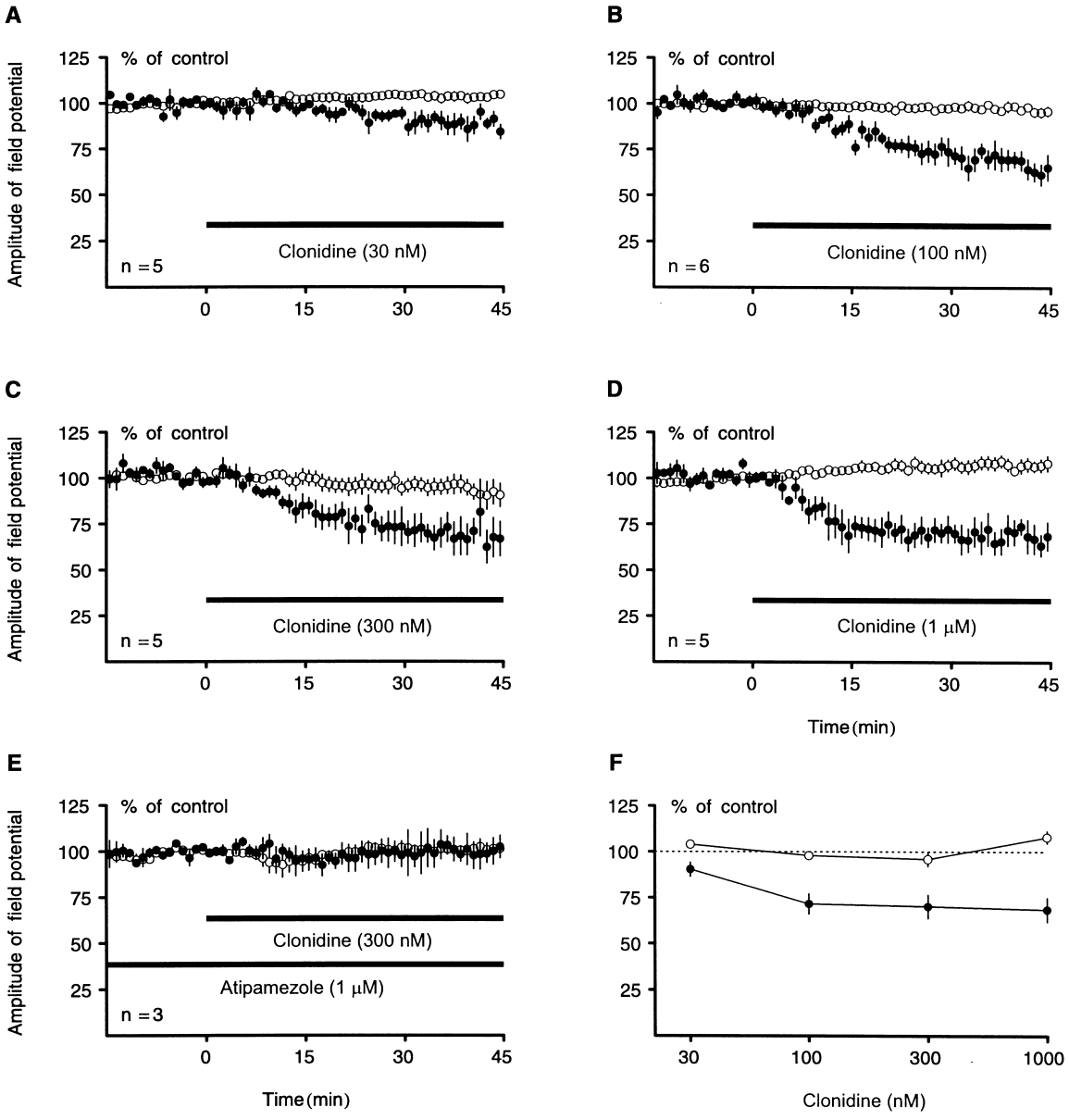

Fig. 7. Clonidine dose-dependently depressed the late potential (closed circles) but not the early potential (open circles). (A–D) Mean time courses of inhibition by different concentrations of clonidine (30–1000 nM horizontal bars) are shown. (E) In the presence of atipamezole (1 μM), clonidine (300 nM) failed to depress the late potential. (F) The dose–response curves for clonidine are shown.

common features with the presently described polysynaptic Aδ-fibre-evoked potential in that both signals are abolished by CNQX, strychnine and bicuculline (Yoshimura and Nishi, 1995). However, the polysynaptic Aδ-fibre-evoked field potential is probably excitatory and its long latency cannot be explained by disynaptic transmission but by oligosynaptic transmission with three to four intercalated neurones. If the late field potential should not reflect IPSPs, its sensitivity to bicuculline and strychnine could be explained by removal of a tonic disinhibition. For example, GABAergic or glycinergic interneurones could tonically inhibit inhibitory neurones that use another inhibitory transmitter. GABAergic terminals presynaptic to enkephalinergic cell bodies and terminals have been found in laminae I and II (Liu et al., 1992) and the release of enkephalin-like material from the spinal cord is inhibited by GABA (Cesselin et al., 1984), suggesting that tonic disinhibition exists in spinal dorsal horn.

Spinal application of bicuculline or strychnine induced allodynia in behaving animals (Onaka et al., 1996) suggesting that excitation of primary afferent Aβ-fibres may gain access to nociceptive pathways in spinal cord. Here, Aδ-

fibre-evoked responses were not facilitated but strongly suppressed, further supporting that these afferents are not responsible for the Aβ-fibre-mediated allodynia.

4.3. An in vitro screening method for potential spinal analgesics

The spinal actions of neuroactive drugs including spinal analgesics can be assessed by a number of well established techniques that range from intrathecal injections in awake, otherwise drug-free animals or humans (Yaksh, 1981) to rapid drug application to excised membrane patches of spinal neurones (Jonas et al., 1998). Evidence exists that cell dialysis has precluded detection of the postsynaptic effects of morphine in whole-cell patch-clamp recordings (Schneider et al., 1998), a problem that is not existent in studies using extracellular recordings. The field potentials studied here are evoked by Aδ-fibres that comprise functionally different classes of sensory afferents including high- and low-threshold mechanoreceptors and cold receptors (Leem et al., 1993). Thus, Aδ-fibre-evoked field potentials are probably not purely nociceptive signals. In contrast to reflex studies the presently characterized field potentials cannot be confounded by effects on the motor system. All slices were obtained from rats more that were at least 18 days old, making developmental influences unlikely (Fitzgerald and Jennings, 1999). We therefore propose that the present in vitro model of spinal nociception may be useful to assess effects of neuroactive drugs on mono- and polysynaptically Aδ-fibre-evoked activity in the mature spinal dorsal horn.

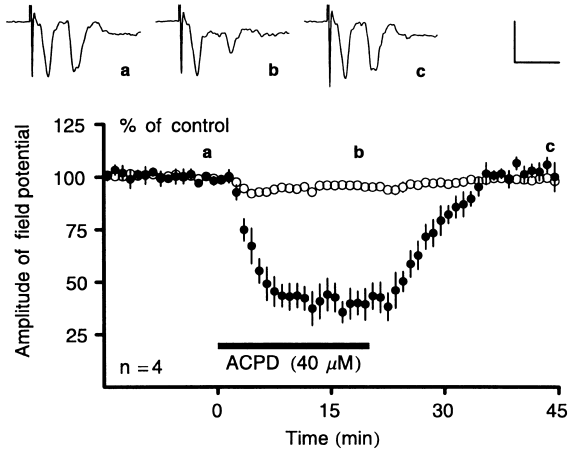

Fig. 8. Bath application of the group I and II metabotropic glutamate receptor agonist, (1*S*,3*R*)-ACPD (40 μM), rapidly and strongly depressed the late potential (closed circles) while leaving the early potential (open circles) almost unaffected. These effects were completely reversible at wash-out. Above the graph, original traces of field potentials recorded immediately before application of (1*S*,3*R*)-ACPD (trace a), after wash-in (trace b) and after wash-out of (1*S*,3*R*)-ACPD (trace c) are shown (calibration bars: 500 μV, 10 ms).

4.4. Selective depression of the polysynaptic spinal field potential by morphine and clonidine

4.4.1. Morphine

Morphine acts predominantly on μ-opiate receptors (Adams et al., 1994) that are abundant in the superficial laminae of the dorsal horn (Besse et al., 1990). μ-Opiate receptors, however, are also present in deeper laminae of the dorsal horn and in the ventral horn (Besse et al., 1990; Gouarderes et al., 1991; Ding et al., 1996). Rhizotomy studies (Besse et al., 1990) and single cell recordings (Zieglgänsberger and Bayerl, 1976; Yoshimura and North, 1983; Schneider et al., 1998; Kohno et al., 1999) suggest that μ-opiate receptors are located on both intrinsic spinal neurones

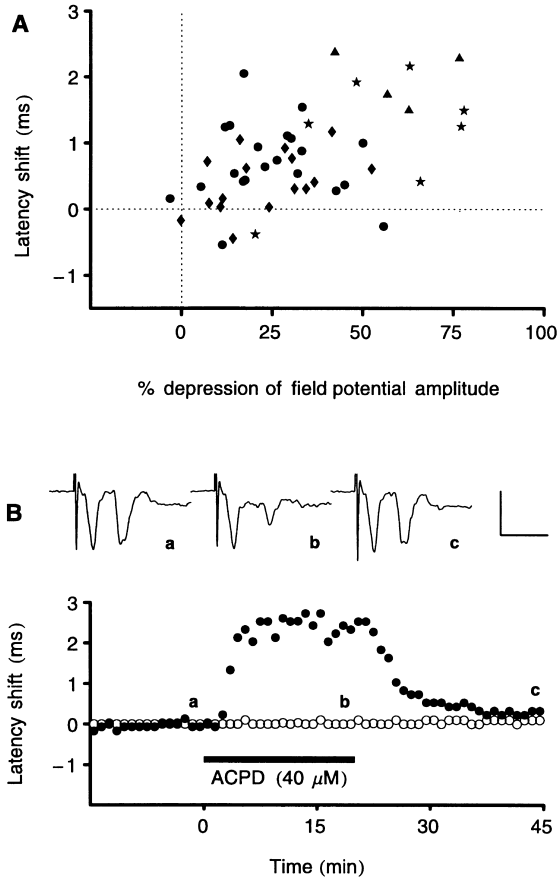

Fig. 9. The drug-induced amplitude depression of the late potential was positively correlated with a shift to longer peak latencies of the late potential. (A) Each symbol represents the depression of the amplitude of the late potential and the corresponding latency shift after application of a drug in a single experiment. The coefficient of correlation between amplitude depression and latency shift was 0.59. (circle: clonidine 30–1000 nM; diamond: morphine 1–10 μM; triangle: (1*S*,3*R*)-ACPD 40 μM; star: after lowering the Ca^{2+} concentration to 2.4 mM). (B) Results from a single experiment with application of (1*S*,3*R*)-ACPD (40 μM) are shown. (1*S*,3*R*)-ACPD induced a strong and fully reversible shift in latency of the late potential (closed circles) but not the early potential. Above the graph, original traces of field potentials recorded immediately before application of (1*S*,3*R*)-ACPD (trace a), after wash-in (trace b) and after wash-out of (1*S*,3*R*)-ACPD (trace c) are shown (calibration bars: 500 μV, 10 ms).

and central terminals of primary afferents. The relative distribution of μ-opiate receptors on terminals of Aδ- and C-fibres, however, is not known.

Extracellularly recorded Aδ-fibre-evoked responses of single dorsal horn cells were depressed by intravenous morphine (Le Bars et al., 1976). In vitro whole cell patch clamp recordings revealed that μ-opioids depressed presumably monosynaptic Aδ-fibre-evoked EPSCs in laminae I and II neurones. This depression was significantly smaller than that of miniature EPSCs suggesting that μ-opioid receptor agonists may preferentially block synaptic transmission of spinal interneurones (Kohno et al., 1999). It has been proposed that morphine preferentially affects polysynaptic potentials that are dependent on spatial and temporal summation (Zieglgänsberger and Bayerl, 1976). The present study supports this notion as application of morphine dose-dependently and selectively depressed the polysynaptic but not the monosynaptic potential.

In contrast to the mixed effects on the responses of neurones in superficial spinal dorsal horn (Sastry and Goh, 1983; Magnuson and Dickenson, 1991), neuronal responses in deeper laminae were consistently depressed by morphine (Zieglgänsberger and Bayerl, 1976; Sastry and Goh, 1983; Magnuson and Dickenson, 1991). The depression of the late field potential by morphine in the present study might be due to a polysynaptic pathway passing through or being modulated by neurones in deeper laminae.

The potency of morphine in the present study was about one-tenth of the potency of morphine on dorsal root-evoked ventral root potentials (Faber et al., 1997). This may be explained by the presence of μ-opiate receptors in the ventral horn that exert an additional inhibition. Another factor influencing the potency of morphine is the extracellular Ca^{2+} concentration that was elevated in our experiments. Morphine and clonidine inhibit transmitter release at least partially by blocking voltage gated presynaptic Ca^{2+} channels, and this effect is reduced at elevated extracellular Ca^{2+} concentrations (Araujo and Collier, 1987). The efficacy of μ-opiate receptor agonists ranges between 30 and 100% (Glaum et al., 1994; Ossipov et al., 1997; Kohno et al., 1999). Here, the maximal depression by morphine of polysynaptic Aδ-fibre evoked field potentials was about 30%.

In conclusion, our results suggest that morphine depresses Aδ-fibre-mediated responses mainly by acting on μ-opiate receptors that are located on spinal interneurones and not on central terminals of primary afferent Aδ-fibres.

4.4.2. Clonidine

Clonidine is a widely used spinal analgesic (Eisenach et al., 1996, 2000; Fairbanks et al., 2000). The analgesia seems to be mediated mainly through activation of α_{2A}-receptors (Stone et al., 1997) that are abundant on terminals in laminae I and II of the dorsal horn of the spinal cord (Stone et al., 1998). Rhizotomy studies (Stone et al., 1998) and the presence of α_{2A}-mRNA in dorsal root ganglion cells (Nicholas et al., 1993) suggest that α_{2A}-adrenoceptors are located on central terminals of primary afferent nerve fibres. In addition, there are also intrinsic lamina I and II cells that express α_{2A}-adrenoceptors (Nicholas et al., 1993).

Extracellular single unit recordings have shown that nociceptive responses of spinal dorsal horn neurones are depressed by spinal application of α_2-adrenoceptor agonists (Fleetwood-Walker et al., 1985; Hamalainen and Pertovaara, 1995). Here, clonidine dose-dependently and selectively depressed the polysynaptic but not the monosynaptic Aδ-fibre-evoked responses. This effect is very similar to that observed with morphine and possible explanations are as discussed above for morphine. The potency of clonidine to depress polysynaptic Aδ-fibre-evoked responses was comparable to its potency to attenuate dorsal root-evoked ventral root potentials (Faber et al., 1997).

Most comparative studies found that clonidine is more potent than morphine in depressing nociceptive responses, the potency ratio varying between 4:1 and 60:1 (clonidine/morphine) on a molar basis (Ness and Gebhart, 1989; Faber et al., 1997; Ossipov et al., 1997). Consistently, we found a potency ratio of about 30:1 (clonidine/morphine).

4.5. The metabotropic glutamate receptor agonist (1S,3R)-ACPD depresses the polysynaptic potential

Up to now, eight different metabotropic glutamate receptors (mGluRs) have been identified that are assigned to groups I–III (Pin and Duvoisin, 1995). (1*S*,3*R*)-ACPD acts as an agonist on group I and group II mGluRs that are present on cell bodies and terminals in laminae I–III of spinal dorsal horn (Jia et al., 1999). Spinal mGluRs may be involved in both induction of hyperalgesia and analgesia. Activation of spinal mGluRs may be necessary for the development of hyperalgesia (Meller et al., 1993) but intrathecal application of (1*S*,3*R*)-ACPD does not consistently produce nociceptive behaviour (Fisher and Coderre, 1996). While (1*S*,3*R*)-ACPD potentiates the responses elicited by AMPA and NMDA in isolated spinal cord dorsal horn neurones (Cerne and Randic, 1992), nociceptive responses of spinothalamic tract neurones are not affected by (1*S*,3*R*)-ACPD (Palecek et al., 1994). Nociceptive discharges of spinal dorsal horn neurones were inhibited by (1*S*,3*R*)-ACPD in animals with a peripheral inflammation but were potentiated in control animals (Stanfa and Dickenson, 1998). In the presence of bicuculline and strychnine but not in normal recording solution, (1*S*,3*R*)-ACPD induced a long term depression of Aδ-fibre-evoked responses in superficial spinal dorsal horn neurones (Chen et al., 2000). The present results confirm that in normal recording solution, monosynaptic Aδ-fibre-evoked responses are not much affected. However, polysynaptic Aδ-fibre-evoked responses were found to be strongly and reversibly inhibited by bath application of (1*S*,3*R*)-ACPD.

5. Conclusions

The present model allows to simultaneously record mono- and polysynaptic Aδ-fibre-evoked field potentials in superficial spinal dorsal horn and to gauge the relative importance of the antinociceptive actions of spinal analgesics at these two different sites. The classical spinal analgesics morphine and clonidine were found to preferentially modulate polysynaptic versus monosynaptic transmission. This model may be useful to study mechanisms of Aδ-fibre-mediated analgesia and hyperalgesia in superficial spinal dorsal horn.

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