Spinal Effects of Bicuculline: Modulation of an Allodynia-Like State by an A1-Receptor Agonist, Morphine, and an NMDA-Receptor Antagonist

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Reeve, Alison J., Anthony H. Dickenson, and Nicola C. Kerr. Spinal effects of bicuculline: modulation of an allodynia-like state by an A1-receptor agonist, morphine, and an NMDA-receptor antagonist. J. Neurophysiol. 79: 1494–1507, 1998. Single-unit recordings were made in the intact anesthetized rat of the responses of dorsal horn neurons to C-, Aδ-, and Aβ-fiber stimulation. The postdischarge and windup responses of the same cells along with responses to innocuous stimuli, prod and brush, were also measured. The effects of (−)-bicuculline-methobromide (0.5, 5, 50, and 250 μg) were observed on these neuronal responses. The C- and Aβ-fiber-activated responses were facilitated significantly in a dose-dependent manner. The input was facilitated, but as the final overall response was not increased by the same factor, windup appeared to be reduced. However, postdischarge, resulting from the increase in the excitability produced by windup, tended to be facilitated. After doses of ≥5 μg bicuculline, stimulation at suprathreshold Aβ-fiber-evoked activity caused enhanced firing, mainly at later latencies corresponding to Aδ-fiber-evoked activity in normal animals. Few cells responded consistently to brush and so no significant change was observed. Responses evoked by innocuous pressure (prod) were always observed in cells that concurrently responded to electrical stimulation with a C-fiber response. This tactile response was facilitated significantly by bicuculline. The effects of N&-cyclopentyladenosine (N&-CPA), an adenosine A1-receptor agonist, was observed after pretreatment with 50 μg bicuculline, as were the effects of morphine and 7-chlorokynurenic acid (7-Ck). N&-CPA inhibited prod, C- and Aδ-fiber-evoked responses as well as the initial and overall final response to the train of C-fiber strength stimuli. Inhibitions were reversed with 8(p-sulphophenyl) theophylline. Morphine, the mu-receptor agonist, also inhibited the postbicuculline responses to prod, C-, and Aδ-fiber responses and initial and final responses to a train of stimuli. Inhibitory effects of morphine were reversed partly by naloxone. 7-Ck, an antagonist at the glycine site on the N-methyl-D-aspartate-receptor complex, inhibited the responses to C- and Aδ-fiber-evoked activity as well as prod. The postdischarges were inhibited by this drug. Again both the initial and overall responses of the cell were inhibited. To conclude, bicuculline caused an increase in the responses of deep dorsal horn cells to prod, Aδ-fiber-evoked activity, increased C-fiber input onto these cells along with the appearance of responses at latencies normally associated with Aδ fibers, but evoked by suprathreshold Aβ-fiber stimulation. These alterations may be responsible for some aspects of the clinical phenomenon of allodynia and hyperalgesia. These altered and enhanced responses were modulated by the three separate classes of drugs, the order of effectiveness being 7-Ck, N&-CPA, and then morphine.

INTRODUCTION

Somatosensory inputs into the dorsal horn of the spinal cord are influenced by local inhibitory controls (Melzack and Wall 1965). A key control is likely to be the inhibitory amino acid, γ-aminobutyric acid (GABA).

GABA and glycine, as well as their synthetic enzymes, are located in dorsal horn interneurons (Barber et al. 1978; Hunt et al. 1981; Magoul et al. 1987; McLaughlin et al. 1975; Powell and Todd 1992; Spike and Todd 1992; Todd 1990; Todd and McKenzie 1989; Todd and Sullivan 1990). GABA may exert presynaptic controls via axo-axonal synapses onto primary afferent terminals (Alvarez et al. 1992; Barber et al. 1978; Bernardi et al. 1995; Hunt et al. 1981; Magoul et al. 1987; Spike and Todd 1992) or postsynaptic inhibitions via axo-dendritic connections (Alvarez et al. 1992; Baba et al. 1994; Magoul et al. 1987; Powell and Todd 1992). In keeping with these studies, the dorsal horn receptors for GABA are located both on primary afferent terminals and at postsynaptic locations (Bowery et al. 1987; Desarmenien et al. 1984; Price et al. 1987).


The roles of these controls have attracted recent interest since spinal levels of GABA decrease after spinal injury (Demediuk et al. 1989; Martiniak et al. 1991; Zhang et al. 1994) and peripheral nerve damage (Castro-Lopes et al. 1993), which may result in a miscoding of afferent information (Hao 1992a,b; Roberts et al. 1986; Woolf 1981; Yaksh 1989). This may underlie the phenomenon of allodynia, common in neuropathic pain states (Bennett 1994), where innocuous stimuli are perceived as painful. Spinal application of bicuculline and strychnine (the glycine receptor antagonist) have been shown to produce behavior indicative of nociception in response to tactile stimulation in normal rats (Roberts et al. 1986; Yaksh 1989). In addition, increased touch evoked activity of motoneurons after administration of bicuculline and strychnine in vivo has been reported, providing evidence for low-threshold activation of the nociceptive reflex under these conditions (Sivilotti and Woolf 1994). A recent study has reported that spinal strychnine produces enhanced neuronal responses to noxious
inputs, primarily an enhanced response to hair deflection, in anesthetized cats. These are reduced by excitatory amino-acid–receptor antagonists (Sorkin and Puig 1996).

The control of allodynia in patients can be refractory to conventional analgesics (see Bennett 1994; Fields and Rowbotham 1994; Portenoy et al. 1990). Yaksh (1989) has studied extensively the behavioral model of allodynia using antagonists to inhibitory amino acids; the induced agitation to tactile stimuli was found to be modulated by N-methyl-D-aspartate-receptor (NMDA) antagonists but not by morphine (Yaksh 1989). In addition, this behavior also can be reduced by spinal application of adenosine receptor agonists (Sosnowski and Yaksh 1989).

To investigate the consequences of spinal bicuculline on neuronal activity, which may represent allodynia in an awake animal, we have studied the response of wide dynamic range neurons in vivo. Peripheral electrical and tactile stimulation were employed to determine the effects of the spinally given GABA_A-antagonist, bicuculline, on the Aβ- and Aδ-, and C-fiber- and natural–evoked responses of these cells. Windup and postdischarge also were studied.

We then investigated the effects of drugs acting at three separate receptor systems, the mu opioid, the NMDA, and the adenosine A1-receptor, based on the studies of Yaksh and colleagues (Sosnowski and Yaksh 1989; Yaksh 1989) to evaluate their potential therapeutic use.

**METHODS**

**Surgery**

Male Sprague-Dawley rats (200–220 g) were anesthetized with halothane in a 66% N2O-33% O2 mixture. The animal then was held in ear bars in a stereotaxic frame, and a laminectomy performed over lumbar segments L1–L3, the cord clamped rostral and caudal to the laminectomy.

**Experimental procedure**

Single-unit extracellular recordings of dorsal horn cells were made using a parylene-coated tungsten electrode. The responses to natural stimuli were established first using nonnoxious brush and prod and noxious pinch. The test regime, consisted, first, of measurement of the responses to controlled innocuous prod, applied by a circular blunt rod allowed to rest over the receptive field (5-mm diam; 4 N/cm2) and then brush with a small paintbrush (10 s each), followed by quantification of the responses evoked by electrical stimuli. One set of tests was made every 10 min. Transcutaneous electrical stimulation of the peripheral receptive field was used to evoke Aβ-, Aδ-, and C-fiber responses in the neurons that could be separated on the basis of latency and threshold. Poststimulus histograms (2-ms bins) were constructed from trials consisting of a train of 16 stimuli at 0.5 Hz with a 2-ms wide pulse. The Aδ- and C-fiber–evoked responses were produced by stimulation at three times the C-fiber threshold (the lowest current required for an action potential in the 90–300-ms latency range). A control poststimulus histogram to this stimulation can be seen in Fig. 1A. The Aβ-fiber–evoked responses were observed separately after stimulation at three times their threshold. The short-latency well-defined response can be seen in Fig. 1C. This form of stimulus will be termed low-intensity rather than Aβ-fiber stimulation because an additional longer latency response was elicited by this stimulation after bicuculline.

After electrical stimulation, the conduction velocity and thresholds of the different peripheral fibers were used to attribute the Aβ-fiber–evoked responses to the 0- to 20-ms latency band, the Aδ fibers to 20–90 ms and the C fibers evoked responses to 90–300 ms. Activity in the 300- to 800-ms range was termed postdischarge, which occurred as windup progressed. Windup, present for every neuron in the study, was quantified by measuring each response of the cell during the latency period 90–800 ms (so including C-fiber–evoked responses and postdischarge) for the trial of 16 stimuli at C-fiber strength (Fig. 1A). This number of stimuli produces consistent maximum windup under these conditions (Dickenson and Sullivan 1987; see Schouenborg and Sjolund 1983). The first response of the neuron was multiplied by 16 and then subtracted from the total response of the cell. This gave a measure of the increased excitability of the cell over the predicted constant response.

We then established the effects of increasing cumulative doses of the GABA_A-receptor antagonist, bicuculline, on the above neuronal responses. Once the optimal dose and time course was established, we tested the effects of N2-cyclopentyladenosine, morphine and 7-chlorokynurenate, given after 50 µg bicuculline pretreatment. The drugs, dissolved in saline, or saline itself as a control, were all applied directly onto the spinal cord, equivalent to an intrathecal administration, in a volume of 50 µl after removal of cerebrospinal fluid. The effects of a particular drug and dose were observed for 40 min. All responses of the neurons after the administration of bicuculline (the dose response for bicuculline and the pretreatment with bicuculline before the subsequent pharmacological manipulations) were expressed as percentages of the initial controls. The effects of the drugs given after bicuculline were expressed as percentage of the last two responses of bicuculline (control) for the dose effects and for the time course as percentages of initial controls ± SEM.

**Data analysis**

Student’s t-test was used to test for significance for the effects of bicuculline versus the initial controls as well as for the effects of the subsequent drugs on the effect of bicuculline. The t-test was paired and two tailed. Drug effects for the time course were analyzed statistically using a one-way analysis of variance with repeated measures, followed by Fisher’s protected least-squares difference (PLSD) post hoc test. Significant level was set at a P value of 0.05.

**Drugs**

N2-cyclopentyladenosine and naloxone were obtained from Sigma; 7-chlorokynurenate and (–)-bicuculline-methobromide from Tocris Cookson; 8(p-sulphophenyl)theophylline from Research Biochemical; and morphine sulphate from Thornton and Ross.

All drugs were made up in saline. 7-chlorokynurenate was buffered to pH 7.3 with the addition of 3 M HCl. (–)-Bicuculline-methobromide was kept in the dark at all times.

**RESULTS**

(–)-Bicuculline-methobromide

The effect of spinal 0.5, 5, 50, and 250 µg bicuculline (0.02–11 mM) was tested on the evoked responses of deep dorsal horn neurons to C-fiber and low-intensity stimulation. The effects of bicuculline on the responses to prod and brush also were measured. The neurons had no ongoing activity will be termed low-intensity rather than Aβ-fiber stimulation be- before drug administration, and bicuculline did not produce any firing of the neurons in the absence of stimulation.

Only one neuron per rat was tested. The effects of all four cumulative doses of bicuculline (0.5, 5, 50, and 250 µg) were tested on nine cells, with an average depth of
FIG. 1. Examples of poststimulus histograms showing the cumulative response of 2 single neurons to a train of 16 stimuli. A: responses of 1 neuron to a train of 16 stimuli produced by stimulation at 3 times the C-fiber threshold (3.6 mA). Evoked responses due to activation of different fiber types are separated on the basis of latency; the Aβ responses between 0 and 20 ms, Aδ fibers between 20 and 90 ms, C fibers between 90 and 300 ms, and postdischarge between 300 and 800 ms. B: responses evoked by suprathreshold C-fiber stimulation after 50 μg bicuculline for the same neuron as in A. Note that the distinction between the responses to different fibers is lost after bicuculline. C: responses evoked at 3 times the Aβ-fiber threshold, low-intensity stimulation (0.4 mA), for another neuron, again with 16 stimuli. D: response to low-intensity stimulation after 50 μg bicuculline of the neuron in C. Note the marked firing in the latency band normally attributed to the Aδ-fiber evoked response after bicuculline.

812 ± 55 μm from the surface of the spinal cord. The effect of 5 μg was observed on eleven other cells (making a total of 20 neurons) with an average depth of 826 ± 46 μm. Bicuculline (50 μg) was studied on a further 43 neurons with an average depth of 811 ± 28 μm, where one of the three modulatory agents subsequently was tested on the bicuculline-evoked changes. Thus overall, 52 cells were studied with this dose, shown by other studies
(Green and Dickenson 1997) to be selective for the GABA$_A$-receptor.

The C-fiber–evoked response was facilitated by bicuculline in a dose-dependent manner, so that the responses were 90 ± 7, 104 ± 7, 114 ± 7, and 166 ± 44% of control values for 0.5, 5, 50, and 250 μg, respectively, with the effects of 50 and 250 μg being significant ($P < 0.05$; Fig. 2A). The A$\delta$-fiber–evoked responses, evoked at C-fiber stimulation strengths, also were facilitated in a dose-dependent manner with the effects of ≥5 μg being significant. The degree of facilitation was far greater for the A$\delta$ responses than the C-fiber–evoked responses, being 99.7 ± 10%, 142 ± 12% ($P = 0.002$), 261 ± 22% ($P < 0.0001$), and 305 ± 60% ($P = 0.003$) of control respectively. An example of the enhancement of the A$\delta$- and C-fiber latency evoked activity produced by bicuculline is in Fig. 1B. With low-intensity stimulation (3 times the A$\beta$-fiber threshold), after doses of ≥5 μg bicuculline, dose-dependent increases in firing at latencies after the main A$\beta$-fiber latency band, primarily in the A$\delta$ range, was observed (Fig. 1, C and D). The extent of these novel responses produced by low-intensity stimulation, were variable between neurons (20 and 200 action potentials...
FIG. 2. Dose-response relations for bicuculline. A: effects of 0.5, 5, 50, and 250 μg bicuculline on the mean response of the population of dorsal horn neurons to electrically evoked Aβ-, Aδ-, and C-fiber-evoked activity (16 stimuli, 0.5 Hz, 2-ms-wide pulse). B: effects on postdischarge and windup produced by the same stimulation. C: effects of bicuculline on the nonnoxious brush and prod responses of the same population of neurons. *P = 0.05, compared with control values. n values range from 9 to 52, mean ± SEM.

FIG. 3. Effects of bicuculline (0.5, 5, 50, and 250 μg) on the initial (input) and final response in a train of 16 stimuli at C-fiber suprathreshold stimulation. Results are given expressed as percentages of the control values before bicuculline. n values range from 9 to 52.

per trial) but no cell ever exhibited any consistent firing after 20 ms without bicuculline.

The effects of bicuculline on the measures of increased excitability, namely postdischarge and windup, were complex and required detailed analysis. The predominant effect of bicuculline was to increase the response of the neurons to the initial C-fiber stimulus in the train. This increased to 109 ± 16, 168 ± 12, 253 ± 24, and 603 ± 231% of control (0.5, 5, 50, and 250 μg of bicuculline), respectively. However, the same doses produced a smaller increase in the overall final responses which were 96 ± 6, 113 ± 9, 126 ± 7, and 264 ± 110% of control respectively. Because windup is the difference between the initial and the overall response, these much greater increases in the initial response produced an apparent decrease in windup (67 ± 8, 80 ± 10, 73 ± 11, and 77 ± 25% of control respectively with the 4 doses) all significant (Figs. 2B and 3). As can be seen in Fig. 4A, what appeared to be a reduction in windup after bicuculline was simply due to a shift in the baseline. The direct measure of increased excitability, namely the postdischarge of the cells, although reduced by 0.5 μg bicuculline (68 ± 14% of control) was increased to 121 ± 24, 153 ± 20 and 195 ± 55% of control, respectively, with the higher doses. Due to variability, only the effects of 50 μg bicuculline was significant on the postdischarge responses (P = 0.03).

The short-latency response to low-intensity stimulation did not show windup after any dose of bicuculline (Fig. 4B).

Although all neurons responded to pinch and prod, only a few neurons had prominent and reliable responses to brush. In neurons where there was a consistent brush response, this showed no significant change from controls with any of the doses of bicuculline (Fig. 2C). The brush evoked activity was 91 ± 36% of control after 0.5 μg of bicuculline, and increasing the doses to 5 and 50 μg gave responses to brush of 101 ± 20, 123 ± 17, and 120 ± 46% of control (n values of 8, 17, and 5, respectively).

On the other hand, vigorous responses to innocuous prod were observed for all neurons in the study. Prod evoked activity was facilitated by all doses of bicuculline, although with the highest dose of 250 μg the response showed less facilitation than with 5 and 50 μg bicuculline; thus 0.5, 5,
to 120 min). These latter periods corresponded to the times of application of the subsequent drugs.

The responses to prod were significantly facilitated in the first ($F_{1,86} = 11.089$, PLSD Fisher's test, $P = 0.001$) and second blocks ($F_{1,84} = 11.822$, $P = 0.0009$), and the overall time course also was increased significantly ($F_{1,262} = 24.544$, $P < 0.0001$; Fig. 5B). The C-fiber-evoked responses also were increased significantly in the first and second blocks ($F_{1,78} = 5.239$, $P = 0.02$; $F_{1,78} = 4.58$, $P = 0.03$, respectively), and, again, when the entire time course was taken into account, the C-fiber-evoked responses were facilitated significantly compared with controls ($F_{1,250} = 11.59$, $P = 0.0008$; Fig. 5A). Postdischarge and windup were not significantly changed from controls in any block of the time course.

The $\alpha$-fiber-evoked activity was facilitated significantly in every time block ($F_{1,78} = 45.118$, $P < 0.0001$; $F_{1,78} =

### Time course

On the basis of the preceding results, the dose of 50 $\mu$g was chosen for the subsequent pharmacological studies because it produced significant effects on all measures except for the $A\beta$ responses. At this stage, it was necessary to determine the time course of the enhanced effects of bicuculline. A separate population of 10 deep dorsal horn neurons (depth 804 ± 71 $\mu$m) therefore was studied after this dose of intrathecal bicuculline (50 $\mu$g). This then was followed by 50 $\mu$l of intrathecal saline, as a control for the modulatory drugs to be tested. The neuron was followed for a further ninety minutes (Fig. 5, A and B). The overall time course was evaluated statistically both as a whole and as separate blocks of 40 min (from 0 to 40 min, 41 to 80 min, and 81

![Figure 4](image1.png)

**Fig. 4.** Effects of bicuculline on windup. Examples are given of the response of a single neuron to a train of 16 stimuli, given at 0.5 Hz with a 2-ms-wide pulse, plotted as action potentials against stimulus number. A: response to C-fiber suprathreshold stimulation at C-fiber latencies and beyond (90–800 ms) before and after the intrathecally applied administration of bicuculline (5 and 50 $\mu$g). B: response of the same neuron to low-intensity stimulation. Here responses from 0 to 800 ms are plotted. Note that even after bicuculline, no windup is produced by this form of stimulation.

50, and 250 $\mu$g bicuculline resulted in responses of 142 ± 39, 218 ± 40, 314 ± 42, and 151 ± 47% of control respectively, with the effects of 5 and 50 $\mu$g bicuculline being significant ($P < 0.005$).

The $\alpha$-fiber-evoked activity was facilitated significantly in every time block ($F_{1,78} = 45.118$, $P < 0.0001$; $F_{1,78} =

![Figure 5](image2.png)

**Fig. 5.** Time course of the responses of a population of dorsal horn neurons to 50 $\mu$g bicuculline applied at time 0, followed by 50 $\mu$l of saline after 40 min. A: responses to $A\beta$-, $A\alpha$-, and C-fiber-evoked activity (the latencies for these responses are given in METHODS). B: responses of post-discharge, windup, brush, and prod of the same cells. Overall time course was changed significantly from controls for the responses to prod, $A\beta$-, $A\alpha$-, and C-fiber-evoked responses. Note that the effects of bicuculline persist for ≤2 h ($n = 9–10$).
TABLE 1. Summary of the effects of 7-CK, N\textsuperscript{6}-CPA and morphine after bicuculline

<table>
<thead>
<tr>
<th>Cell Response</th>
<th>7-CK (50 μg)</th>
<th>N\textsuperscript{6}-CPA (50 μg)</th>
<th>Morphine (1 μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prod</td>
<td>6 ± 5</td>
<td>13 ± 6</td>
<td>42 ± 19</td>
</tr>
<tr>
<td>C fibers</td>
<td>52 ± 17</td>
<td>61 ± 20</td>
<td>73 ± 13</td>
</tr>
<tr>
<td>Aβ fibers</td>
<td>18 ± 6</td>
<td>41 ± 2</td>
<td>43 ± 14</td>
</tr>
<tr>
<td>Aδ fibers</td>
<td>62 ± 19</td>
<td>78 ± 24</td>
<td>82 ± 10</td>
</tr>
<tr>
<td>Postdischarge</td>
<td>32 ± 13</td>
<td>15 ± 5</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>Initial response</td>
<td>21 ± 8</td>
<td>34 ± 12</td>
<td>41 ± 10</td>
</tr>
<tr>
<td>Overall response</td>
<td>44 ± 14</td>
<td>69 ± 20</td>
<td>66 ± 10</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

The mean maximal modulatory effects of the N-methyl-D-aspartate-receptor antagonist, 7-chlorokynurenate (7-CK), the A\textsubscript{1}-adenosine receptor agonist, N\textsuperscript{6}-cyclopentyladenosine (N\textsuperscript{6}-CPA), and the mu-opioid receptor agonist, morphine, expressed as percent of the final effects of 50 μg bicuculline pretreatment. The results of the postdischarge shown are for the inhibited cells only. Responses in parentheses show the overall response for both inhibited and facilitated cells. Values are means ± SEM.

25.986, \( P < 0.0001 \); \( F_{1,72} = 9.204, P = 0.003 \), respectively. and, again, the entire time course was facilitated significantly (\( F_{1,248} = 72.570, P = 0.0001 \); Fig. 5A). The Aβ-fiber latency evoked responses after low-intensity stimulation were facilitated significantly as an overall time course (\( F_{1,248} = 19.966, P < 0.0001 \)) with the first two blocks also being facilitated significantly (\( F_{1,78} = 10.733, P = 0.001 \); \( F_{1,78} = 6.17, P = 0.01 \), respectively).

Receptive fields of the neurons

The receptive fields of all neurons were on the ipsilateral hindpaw. The receptive fields for brush, prod, and pinch were checked by applying the appropriate stimuli to the paw and observing the evoked firing of the neuron. However, because there was no obvious change in the size of the receptive field to any of the stimuli, after any dose of bicuculline, the sizes were not quantified.

N\textsuperscript{6}-cyclopentyladenosine

In all drug studies, the drugs were applied after a 40-min pretreatment with 50 μg intrathecal bicuculline, and the drug effects expressed as percentages (± SEM) of the final two values after bicuculline. A summary is given in Table 1.

The effects of spinal N\textsuperscript{6}-cyclopentyladenosine (N\textsuperscript{6}-CPA), an A\textsubscript{1}-adenosine agonist, at doses of 5 and 50 μg, were tested on a population of eight neurons with an average depth of 836 ± 85 μm. Each dose was followed for 40 min, after which 250 μg 8pSPT, an A\textsubscript{2}-adenosine receptor antagonist was applied. The postbicuculline prod and the C-fiber–evoked responses were inhibited significantly by N\textsuperscript{6}-CPA and the effects were reversed by 8pSPT (\( P < 0.05 \); Fig. 6, A and B). N\textsuperscript{6}-CPA significantly inhibited (\( P < 0.05 \)) the postbicuculline Aβ-fiber latency responses of six of the eight cells, but in two cells, the responses remained facilitated. However, when compared with the initial controls, the Aδ-fiber responses after N\textsuperscript{6}-CPA, although markedly reduced, were still marginally at levels above the baseline response (see Fig. 6A).

The bicuculline-enhanced Aβ-fiber latency responses were significantly inhibited by both 5 and 50 μg N\textsuperscript{6}-CPA, and the reversal with 8pSPT was also significant (\( P < 0.05 \)).

The enhanced firing evoked by low-intensity stimulation after bicuculline, occurring in the latency usually associated with Aδ-fibers (20–90 ms), also was decreased or abolished by N\textsuperscript{6}-CPA. The postdischarge of three cells was facilitated after N\textsuperscript{6}-CPA and inhibited for five neurons.

After bicuculline there was an increased initial and final response of the cells compared with controls. N\textsuperscript{6}-CPA (5 and 50 μg) decreased the augmented initial response to 58 ± 12 and 34 ± 12% of control, respectively (Fig. 7, A and B). The overall final response of the cells was decreased by N\textsuperscript{6}-CPA, but this reached a plateau with 5 μg, so the effects were 68 ± 16 and 69 ± 20% of control values, respectively, for 5 and 50 μg. These inhibitions were reversed by 8pSPT.

Morphine

The effects of morphine (0.25 and 1 μg) were observed on seven neurons (depth 902 ± 57 μm). The C- and Aβ-
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inhibited but there was no clear dose dependency. Neither response was reversed fully with naloxone (Fig. 9A).

The activity evoked by prod was facilitated after bicuculline (see Fig. 8B), and although morphine tended to inhibit the response to prod, this was only significant after 1 μg morphine ($P = 0.006$; reduction to $42 \pm 19\%$ of control (see Table 1). Reversal with naloxone was not significant.

7-chlorokynurenate

The effects of 7-chlorokynurenate (7-CK) after bicuculline was observed on the responses of seven neurons (depth of $826 \pm 64 \mu m$).

Apart from brush responses ($n = 3$), all other postbicuculline-enhanced responses of the neurons were reduced significantly by both 10 and 50 μg 7-CK (Fig. 10, A and B, Table 1). However, there was a more marked inhibition of some responses compared with others. The rank order of inhibition with 50 μg 7-CK was prod, Aδ, postdischarge, C.

The Aδ-fiber responses evoked at C-fiber stimulation were facilitated by bicuculline and significantly inhibited compared with bicuculline controls by both doses of morphine ($P < 0.02$). The postdischarges of two cells were facilitated by morphine and inhibited for four cells, a similar pattern to that seen with N⁶-CPA, so the overall effect was not significant.

The additional firing seen in the latency range 20–90 ms, after bicuculline, after low-intensity stimulation, was inhibited by 0.25 and 1 μg morphine (Fig. 9, A and B; Table 1). The overall final response of the cell was inhibited but there was no clear dose dependency. Neither response was reversed fully with naloxone (Fig. 9A).
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The intrathecal application of bicuculline onto the exposed spinal cord produced a moderate increase in the \( \alpha \beta \)- and \( \gamma \)-fiber-evoked responses of deep dorsal horn neurons as well as a more dramatic increase in both the firing of these cells at latencies normally associated with \( \alpha \delta \)-fiber responses and to innocuous pressure. Responses to brush were increased by bicuculline but not consistently. It is impossible to determine whether the increased activity, in the latency band usually attributed to the \( \alpha \delta \)-fiber-evoked responses, reflects a prolongation of firing produced by \( \alpha \beta \)-fiber inputs or disinhibition or excitation of true \( \alpha \delta \)-fiber activity. After bicuculline, near-maximal increases were seen with low-intensity stimuli: \( \gamma \)-fiber strength stimulation did not produce any further increase in activity in the \( \alpha \delta \) latency band. In the control

**DISCUSSION**

These electrophysiological results extend the evidence that GABA acts as a modulating neurotransmitter within the dorsal horn of the spinal cord via the activation of the GABA\( \alpha \)-receptor and reinforces the idea that the loss of GABA\( \alpha \)-receptor mediated inhibition can lead to the miscoding of low-threshold information. This may give rise to allodynia, as demonstrated by blocking GABAergic and glycnergic systems in a variety of models (Hao et al. 1992a; Sorkin and Puig 1996; Sosnowski and Yaksh 1989; Yaksh 1989).

FIG. 9. As for Fig. 7, the inhibitory effects of morphine (0.25 and 1 \( \mu \)g) and reversal by naloxone (0.5 \( \mu \)g) on the input and final responses are shown in A (\( n = 7 \)). B: windup. Response of a single neuron to a train of stimuli at \( \gamma \)-fiber stimulation plotted as action potentials against stimulus number (16 in total). Response before pretreatment with bicuculline is shown as the control. Increased response produced by 50 \( \mu \)g bicuculline (BIC) was inhibited partly by morphine.

and \( \alpha \beta \) fibers (Table 1). By way of example, these responses after bicuculline were reduced to \( 6 \pm 5 , 18 \pm 6 , 32 \pm 13, 51 \pm 17 \), and \( 62 \pm 19 \% \) of control with 50 \( \mu \)g 7-CK, respectively (\( P < 0.05 \)).

The activity evoked after bicuculline to low-intensity stimulation occurring in the latency band 20–90 ms was decreased after 10 and almost abolished after 50 \( \mu \)g 7-CK.

Overall, both concentrations of 7-CK inhibited the initial response (35 \( \pm \) 10 and 21 \( \pm \) 8\% of control for 10 and 50 \( \mu \)g 7-CK, respectively) and the final response of the cell (70 \( \pm \) 7 and 44 \( \pm \) 14\% of control for the same doses, respectively; see Fig. 11A). However, it appears that the initial response, as compared with the final response, was more sensitive to the inhibitory effects of 7-CK. It was apparent from the individual windup curves that although 7-CK reduced the initial response, there was a more limited effect on windup (Fig. 11B).

**FIG. 10.** Effect of intrathecally applied 7-chlorokynurenate (7-CK; 10 and 50 \( \mu \)g) after 50 \( \mu \)g bicuculline. A and B: time course of the responses after bicuculline pretreatment and then the effects of 2 doses of 7-CK (10 and 50 \( \mu \)g) on these altered responses, expressed as percentages of initial control responses. Responses to \( \alpha \beta \)-, \( \alpha \delta \)-, and \( \gamma \)-fiber-evoked responses are in A and the postdischarge, windup, brush (\( n = 3 \)) and prod responses are in B (\( n \) values range from 6 to 7 for all other responses).
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and prominent brush responses were few and most cells with con-nections onto presynaptic terminals of A fibers also can be responsible for this phenomenon. We found the proportion of cells exhibiting consistent and prominent brush responses were few and most cells with clear brush responses failed to respond to C-fiber-evoked activity so were not selected for this study on wide-dynamic range neurons. However, cells always responded to innocuous prod, and these responses were found to be facilitated markedly and significantly after bicuculline. This increased activity is probably low threshold because the prod stimulus was entirely nonpainful and would appear to be below the pressure threshold for Aδ nociceptors in the hindpaw described in rats and cats (Garell et al. 1996; Leem et al. 1993). Some Aδ-fibers respond to low-threshold mechanical stimuli (see Besson and Chaouch 1987) and have been suggested to underlie allodynia in humans (Persson et al. 1995). Sensory testing in patients with Von Frey hairs most probably also represents a response to pressure/prod, and punctate as well as dynamic and static allodynia can occur in neuropathic states (Koltzenburg et al. 1994). In humans, allodynia and hyperalgesia are not restricted to any particular body surface. Our study used the glabrous skin of the hindpaw, and so it is not unexpected that prod rather than brush was a more reliable effective low-threshold stimulus for these neurons.

In hairy skin of the cat, after spinally applied strychnine, the response to pressure is increased moderately compared with a much greater elevated brush response. They, like us, found no increase in spontaneous activity of the neurons after application of strychnine (Sorkin and Puig 1996). However, it is quite possible that there is differential control of brush and pressure by glycine and GABA because there is anatomic evidence for a preferential control of Aβ/Aδ-fiber—evoked responses, respectively (Alvarez et al. 1992; Bernardi et al. 1995), which also may relate to differences in somatosensory processing from glabrous compared with hairy skin. In the monkey, bicuculline causes increased spinal neuronal responses to brush and also provokes an ongoing activity and expanded receptive fields (Lin et al. 1996). The differences between the studies may well be due to different species and anesthetics and also the type of skin to which the natural stimuli were applied.

The spinal application of antagonists of the receptors for the inhibitory amino acids, GABA and glycine, have been reported to cause agitation and cardiovascular arousal to nonnoxious stimulation such as hair deflection (Roberts et al. 1986; Sherman and Loomis 1994; Yaksh 1989). Electrophysiological experiments have demonstrated enhancements in the evoked firing patterns of cells produced by both low- and high-threshold stimulation in the presence of antagonists of these two receptor systems (Curtis et al. 1968; Duggan et al. 1981; Game and Lodge 1975; Sivilotti and Woolf 1994). Not only were low-threshold stimuli enhanced to a greater extent than pinch, but low-intensity stimuli could activate the noxious reflex in motoneurons after antagonist treatment (Sivilotti and Woolf 1994). These observed changes have been likened to the phenomenon of alldynia and have been attributed to the loss of inhibitory amino-acid controls within the dorsal horn of the spinal cord (Hao et al. 1992a,b; Sivilotti and Woolf 1994; Yaksh 1989; Yaksh and Harty 1988).

There is ample evidence for a GABA control of low-threshold afferent activity in the spinal cord (see Curtis 1969), but recent immunohistochemical studies have shown that GABAergic terminals make the greatest number of connections onto presynaptic terminals of Aδ-fiber afferents compared with C fibers (Alvarez et al. 1992; Bernardi et al. 1995). Our results would support this although we are unable to exclude possible effects of the drugs on dorsal roots or dorsal root ganglion cells.
Although the postdischarge of the cells, generated by suprathreshold C-fiber stimulation after GABA<sub>A</sub>-receptor antagonism was observed to increase, indicating an increase in excitability to noxious stimuli, paradoxically, the windup of the same cells apparently decreased. This was due to the method of calculation of windup that was measured as the excess firing over the predicted response based on extrapolation of the initial C-fiber-evoked input. Bicuculline produced a marked increase in the initial response of the neurons. As the final overall response was not increased by the same factor, the calculated values for windup, dependent on the ratio of the initial and final overall activity, seemed to be reduced.

There was also an increase in the initial response onto the cell after low-intensity stimulation, but this was not as great as that seen with suprathreshold C-fiber stimuli. The response of cells to low-intensity stimulation did not show a ‘‘windup like’’ response with any dose of bicuculline.

The enhancement of responses of deep dorsal horn neurons caused by spinal antagonism of GABA<sub>E</sub>ergic controls with bicuculline subsequently were modulated pharmacologically by administration of spinally applied adenosine A<sub>1</sub>-receptor agonist, N<sup>6</sup>-CPA, the mu opioid-receptor agonist, morphine, and the NMDA-receptor antagonist, 7-chlororokynurenate. A previous study observed a relatively short duration of action of antagonists of the inhibitory amino acids measuring behavioral agitation (Yaksh 1989). We found that effects plateaued between 20 and 40 min after initial bicuculline administration. However, these effects were still significantly enhanced during a longer time course (≥2 h); this justified our subsequent pharmacological studies on the modulation of the responses. Because all three pharmacological agents were tested with the same paradigm, their relative effectiveness would be independent of the time course of the bicuculline effect.

The A<sub>1</sub>-agonist, N<sup>6</sup>-CPA, inhibited the enhanced A<sub>δ</sub>- and C-fiber responses after bicuculline treatment, along with the postdischarge of the cells. The increased response to prod also was inhibited by N<sup>6</sup>-CPA, and these inhibitions were reversed by the antagonist 8pSPT. The increased firing of cells with low-intensity stimulation, which now occurred at latencies usually associated with the A<sub>δ</sub>-fiber-evoked responses, was dose dependently and almost fully inhibited by N<sup>6</sup>-CPA, and was restored to a lesser extent by 8pSPT.

The increased excitability of the cells, manifested by the postdischarge response, fell into two populations with regard to the direction of effects of N<sup>6</sup>-CPA. A small proportion had facilitated responses, but in the main, this response was inhibited after bicuculline. N<sup>6</sup>-CPA decreased the initial neuronal response of the neurons, which had been increased previously by bicuculline, as well as reducing, to a lesser extent, the overall final response.

Adenosine has well-documented analgesic properties in both animal and human studies via the A<sub>1</sub>-receptor (Belfrage et al. 1995; Karlsten and Gordh 1995; Reeve and Dickenson 1995; see Salter et al. 1993; Sawynok and Sweeney 1989). Animal studies with adenosine analogues (Sosnowski and Yaksh 1989) and recent human studies with adenosine have indicated that adenosine infusion can attenuate allodynia induced either by peripheral stimuli or as a result of peripheral nerve damage (Belfrage et al. 1995; Karlsten and Gordh 1995; Sergerdahl et al. 1995; Sollevi et al. 1995). N<sup>6</sup>-CPA was extremely effective at reducing almost all of the enhanced responses of the neurons after bicuculline, thus providing a possible basis for the use of adenosine in neuropathic states.

The NMDA receptor has been implicated as a possible target in neuropathic pain (see Dickenson 1994a) because its activation underlies hyperalgesia in a number of persistent pain states. The reasons for this are that the receptor is required for windup, whereby C-fiber stimulation produces an amplified and prolonged neuronal response (Davies and Lodge 1987; see Dickenson 1994a; Dubner and Ruda 1992; McMahon et al. 1993; Price et al. 1994). GABA inhibitory neurons may regulate the release of glutamate from primary afferents terminals (Alvarez et al. 1992; Bernardi et al. 1995), so a loss of this GABA<sub>A</sub> control of afferents would result in peripheral stimuli evoking a greater release of glutamate. In fact, this could be the basis for our observation that the response to the first stimulus at C-fiber strength was elevated massively after bicuculline, with a lesser increase in the initial response to low-intensity electrical stimulation. Any reduced control of the release of excitatory neurotransmitters then could result in reorganization/miscoding of inputs, via the recruitment of the NMDA receptor (Ma and Woolf 1995; Yaksh 1989). Supporting this idea are reports that glutamate receptor antagonists decrease hyperesthesia and hyperalgesia (Backonja et al. 1994; Tal and Bennett 1994; Yamamoto and Yaksh 1993) as well as allodynia in humans and in animal models (Backonja et al. 1994; Bennett 1994; Eide et al. 1994; Persson et al. 1995; Sherman and Loomis 1994; Yaksh 1989) and decrease windup like phenomenon in humans with neuropathic pain (Eide et al. 1994; Kristensen et al. 1992).

The effects of the NMDA glycine site antagonist, 7-CK, were used to gauge both the involvement of this receptor in the postbicuculline responses and also to investigate the ability of agents acting at this site to modulate altered nociception (Chapman and Dickenson 1992; Dickenson and Aydar 1991). 7-CK profoundly inhibited all responses of the deep dorsal horn cells to electrically and naturally applied stimuli. The responses to prod were inhibited to the greatest extent, followed by the A<sub>δ</sub>-, postdischarge, C-, and A<sub>δ</sub> fiber-evoked responses. The increased activity at A<sub>δ</sub>-fiber latencies to low-intensity stimulation after bicuculline was abolished by 7-CK. The initial response of the neurons evoked by C-fiber suprathreshold activation was reduced by 7-CK to a greater extent than the cumulative responses, so that windup appeared to be restored. Interestingly, in normal animals, the profile of 7-CK is different in that it has no effect on had facilitated responses, but in the main, this response was inhibited after bicuculline. N<sup>6</sup>-CPA decreased the initial neuronal response of the neurons, which had been increased previously by bicuculline, as well as reducing, to a lesser extent, the overall final response.
glycine antagonist produces neuronal changes indicative of allodynia and hyperalgesia in the cat, both of which are reduced by NMDA-receptor antagonism (Sorkin and Puig 1996), in agreement with our results.

The opioid effectiveness can alter in different pain states (see Dickenson 1994b). A consensus would be that opioids have reduced effectiveness in neuropathic conditions (Arner and Meyerson 1988; Jadad et al. 1992; Portenoy et al. 1990). In particular, it has been reported that opioids do not decrease hypersensitivity attributed to allodynia (Arner and Meyerson 1988; Lee et al. 1994; Lee et al. 1995; Sherman and Loomis 1989) although effects on allodynia have been reported (Desmeules et al. 1993; Eide et al. 1994).

We found that although morphine did weakly inhibit the response to prod, the C- and Aδ-fiber-evoked responses and the initial responses of the cells, it was the least effective out of the three drugs observed. The inhibition by morphine of the postdischarge of the cells was not clear cut in that there were both inhibited and facilitated responses. By far the most noticeable difference between morphine, N6-CPA and 7-CK was the inhibition of the responses evoked by prod, which was inhibited to a greater extent by N6-CPA and 7-CK compared with morphine.

Although the literature is somewhat contradictory, it would appear that NMDA-receptor antagonists and A1-receptor agonists are effective at treating allodynia (Belfrage et al. 1995; Bennett 1994; Karlsten and Gordon 1995; Ma and Woolf 1995; Persson et al. 1995; Sergerdahl et al. 1995; Sherman and Loomis 1994; Sollevi et al. 1995; Tal and Bennett 1994; Yaksh 1989; Yamamoto and Yaksh 1993), whereas opioids are not as effective compared with nociceptive pain in humans and animals (Arner and Meyerson 1988; Lee et al. 1994; Lee et al. 1995; Sherman and Loomis 1994; Yaksh 1989). This is in keeping with our findings in that although morphine was effective, it was not as potent as 7-CK or N6-CPA at decreasing changes evoked by GABAergic disinhibition.

Electrophysiological studies cannot be used to demonstrate allodynia in the sense of experiencing distress and presumably pain to nonnoxious stimulation. However, we do see hyperalgesia, in that the responses to C-fiber-evoked stimulation increases. We also saw an increased response to innocuous prod as well as a change in the evoked firing patterns of deep dorsal horn neurons at both C and low-intensity stimulation. It has been suggested, based on many different models of evoked hyperresponsiveness produced by antagonism of inhibitory mechanisms (Game and Lodge 1975; Roberts et al. 1986; Sosnowski and Yaksh 1989; Yaksh 1989; Yamamoto and Yaksh 1993), high doses of opioids (Woolf 1981; Yaksh and Harty 1988) peripherally induced changes (Ma and Woolf 1995), and spinal ischemia (Hao et al. 1992a), that a loss of ongoing inhibitions results in the misconduct of somatosensory information by deep dorsal horn neurons. The changes in response patterns of deep dorsal horn neurons reported here may reflect mechanisms that lead to hyperalgesia and allodynia in awake animals. Thus our evidence further supports the idea of an important inhibitory role of GABA within the dorsal horn and indicates a basis for the use of NMDA-receptor complex antagonists and adenosine in the control of both allodynia and hyperalgesia as observed in behavioral studies with this and other models (Bennett 1994; Sosnowski and Yaksh 1989; Yaksh 1989).

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