Effects of Clonidine and Baclofen on Prostaglandin $F_2\alpha$– induced Allodynia in Conscious Mice

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Abstract

The intrathecal administration of prostaglandin $F_2\alpha$ to conscious mice resulted in allodynia elicited by non-noxious brushing of the flanks. The maximum allodynia induced by prostaglandin $F_2\alpha$ was observed 10–15 min after intrathecal injection, and the response lasted over the experimental period of 50 min. This allodynia was dose-dependently relieved by the $\alpha_2$-adrenergic agonist clonidine and the GABA$_\alpha$ agonist baclofen. The alleviation by baclofen was reversed by theophylline, but that by clonidine was not reversed. These results demonstrate that the allodynia involves the $\alpha_2$-adrenergic and GABA$_\alpha$ systems but that the modes of association of prostaglandin $F_2\alpha$ with these two systems may be different. Because this allodynia has a clear resemblance to the characteristics of chronic pain in patients with causalgia and reflex sympathetic dystrophy, it may be a useful pain model for chronic pain and provide further evidence for the importance of the spinal noradrenergic and GABA systems in the transmission and treatment of patients with chronic pain.

Key Words: prostaglandin $F_2\alpha$, allodynia, spinal cord, clonidine, baclofen, theophylline

INTRODUCTION

Intrathecal (i.t.) administration of strychnine or a high dose of morphine into rats was reported to induce allodynia, a state of discomfort and pain evoked by innocuous stimuli; the rats showed squeaking, biting, and scratching movements in response to low-threshold stimuli$^{12,17,18}$. The features of the allodynia resemble those of patients suffering from sympathetically maintained pain. Recent studies have suggested that the pharmacology of the system activated in
the pathologic state “alldynia” may differ from that activated under normal circumstances by high-threshold thermal, chemical, and mechanical stimuli. This selective modification is thought to result from the association of several receptor types such as glutamate and adenosine receptors with the spinal system processing these types of information\(^1\). Prostaglandins (PGs) are ubiquitously distributed in virtually all mammalian tissues and organs, and it has been well documented that PGs are involved in various aspects of inflammation including pain\(^1\). We recently demonstrated that i.t. injection of PGF\(_2\)\(\alpha\) induced alldynia in mice, which was reversed by \(\alpha_2\)-adrenergic and A\(_1\)-adenosine agonists\(^4\). Our previous study implied that agents with a capacity to inhibit adenylate cyclase be effective in reducing the PGF\(_2\)\(\alpha\)-induced alldynia. To prove this notion, we examined here the effect of clonidine, an \(\alpha_2\)-adrenergic agonist, baclofen, a GABA\(_\beta\) agonist, and theophylline, a phosphodiesterase inhibitor, on the alldynia.

**MATERIALS AND METHODS**

**Intrathecal administration**

Male ddy-mice weighing \(24 \pm 2\) g were used in this study. The animals were housed under conditions of a 12-h light-dark cycle and a constant temperature of \(22 \pm 2\) °C and \(60 \pm 10\) % humidity. A 27-gauge stainless-steel needle (0.35 mm o.d.) attached to a microsyringe was inserted between the L\(_6\) and L\(_6\) vertebrae by a slight modification of the method of Hylden and Wilcox\(^5\). Drugs in vehicle were injected slowly into the subarachnoid space of conscious mice at \(22 \pm 2\) °C. The volume of the i.t. injection was \(5 \mu\)l. It was previously confirmed by use of Commassie brilliant blue and \(^{3}\)H]PG that the injected solution did not extend to the cervical segments\(^1\).

**Studies on alldynia**

Studies on alldynia were carried out essentially according to the method of Yaksh and Hardy\(^6\). The mice were divided into various groups (n = \(6\)–\(8\)/group). Control mice were given physiological saline (\(5 \mu\)l). Drug-treatment groups were injected with \(5 \mu\)l of vehicle containing various doses of test agents. After injection, each mouse was placed in an individual \(13 \times 8.5 \times 13\) cm Plexiglas enclosure with wood chips on the floor and observed. Alldynia was assessed once every 5 min by light stroking of the flank of the mice with a paintbrush. The alldynia response was ranked as follows: 0, no response; 1, mild squeaking with attempts to move away from the stroking probe; 2, vigorous squeaking evoked by the stroking probe, biting at the probe, and strong efforts to escape. Each mouse was tested for 50 min following i.t. injection.

To evaluate the effects of the agents on alldynia, we assessed the scores over a 50-min period for alldynia, and the values were cumulated and expressed as a percent of the maximum possible score. Thus, the maximum possible score for alldynia per animal was 20.

The animals were used only for one measurement in each experiment. This study was conducted in concordance with the guidelines of the Ethics Committee of the International Association for the Study of Pain\(^2\).

**Drugs**

PGF\(_2\)\(\alpha\) was a generous gift from Ono Central Research Institute (Osaka, Japan). PGF\(_2\)\(\alpha\) was stored in ethanol solution at \(-20\) °C. For injection, an aliquot of the desired stock PGF\(_2\)\(\alpha\) solution was put into a borosilicate tube, and the ethanol was removed by evaporation to dryness under nitrogen gas. Sterile saline was then added to dissolve the PGF\(_2\)\(\alpha\). Clonidine hydrochloride, baclofen, and theophylline were obtained
from Sigma (St. Louis, MO). They were dissolved in sterile saline on the day of the experiment and kept on ice until used. All drugs, including saline, were coded to assure blind testing.

**Statistics**

The statistical analyses were carried out by analysis of variance (ANOVA). Statistical significance (P < 0.05) was further examined with Duncan’s test for multiple comparison.

**RESULTS**

**Effect of i.t. PGF$_{2\alpha}$ on allodynia**

As reported previously\(^9\), the i.t. administration of PGF$_{2\alpha}$ resulted in prominent agitation responses, such as vocalization, biting escape from the probe, to tactile stimuli applied to the flank. Brushing of the face or tactile stimulation of the forepaws did not give any responses, indicating that allodynia appeared limited to the caudal dermatomes of the body. Figure 1 presents the time courses of allodynia evoked by different concentrations of PGF$_{2\alpha}$. Allodynia was evoked by the first stimulus 5 min after i.t. injection and the maximum effect was observed at 10–15 min. The response was long lasting and did not disappear by 50 min. PGF$_{2\alpha}$-treated mice did not display clonic seizure and convolution even at a high dose of 1 $\mu$g/mouse or more. On the other hand, the allodynia was induced at a dose as low as 0.1 pg/mouse, but the i.t. administration of saline had no effect on allodynia, suggesting that PGF$_{2\alpha}$ may be a natural substance participating in allodynia.
Effects of clonidine or baclofen on PGF$_2\alpha$-evoked allodynia

To examine whether agents with a capacity to inhibit adenylate cyclase are effective in reducing allodynia, we investigated the effect of the $\alpha_2$-adrenergic agonist clonidine and the GABA$_A$ agonist baclofen on allodynia caused by PGF$_2\alpha$.

When clonidine or baclofen was coadministered with PGF$_2\alpha$ (1.0 $\mu$g/mouse), the allodynia declined sharply and disappeared by 25 min after i.t. injection. As shown in Fig. 2, when assessed by cumulative scoring during the overall 50-min period, the allodynia caused by 1.0 $\mu$g of PGF$_2\alpha$ was dose-dependently blocked by both clonidine and baclofen. The IC$_{50}$ values of clonidine and baclofen were 0.17 $\mu$g and 13 ng, respectively. The doses of clonidine or baclofen that we used here were lower than those used in most animal studies. The i.t. injection of clonidine or baclofen alone showed no effect on the response to innocuous stimuli. These results demonstrate that the $\alpha_2$-adrenergic and GABA$_A$ systems are involved in the PGF$_2\alpha$-induced allodynia.

Effect of theophylline on inhibition of PGF$_2\alpha$-induced allodynia by clonidine or baclofen

Theophylline is known to inhibit phosphodiesterase enzymes at pharmacologic doses. Therefore we administered this drug to investigate if the antiallodynic effect of clonidine or baclofen was caused by a cAMP-mediated process. Theophylline was injected into the subarachnoid space 15 min before i.t. administration of 1.0 $\mu$g of PGF$_2\alpha$ and indicated doses of clonidine or baclofen. As shown in Table 1, the blockade of PGF$_2\alpha$-induced allodynia by baclofen was completely reversed by theophylline.
Table 1  Effect of theophylline on inhibition of PGF<sub>2α</sub>–induced allodynia by clonidine or baclofen. Theophylline was injected into the subarachnoid space 15 min before i.t. administration of 1.0 μg of PGF<sub>2α</sub> and indicated doses of clonidine or baclofen. The values (mean±S.E.) in each column were compared with the one in the absence of theophylline. The scores of PGF<sub>2α</sub> alone were taken as 100%. Statistical analyses were conducted by Duncan’s test.

<table>
<thead>
<tr>
<th>Dose (ng/mouse)</th>
<th>Alloodynia (% of control)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Theophylline</td>
</tr>
<tr>
<td></td>
<td>(−)</td>
</tr>
<tr>
<td>Clonidine</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>57.0±9.03</td>
</tr>
<tr>
<td>2000</td>
<td>8.6±2.72</td>
</tr>
<tr>
<td>Baclofen</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>15.1±2.72</td>
</tr>
</tbody>
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** P ≤ 0.01

(40 μg/mouse), but that by clonidine was not antagonized. Theophylline alone induced spontaneous agitation in all mice examined but did not evoke allodynia.

**DISCUSSION**

We recently demonstrated that i.t. injection of PGF<sub>2α</sub> induced allodynia in mice, which was dose–dependently relieved by clonidine<sup>9</sup>. In the present study, we showed that baclofen also antagonized the PGF<sub>2α</sub>–induced allodynia (Fig. 2). Receptors for both α<sub>2</sub>–adrenergic and GABA<sub>B</sub> are present on primary afferent terminals in laminae I to IV of the dorsal horn in the spinal cord<sup>10,19</sup>, and are known to have in common their ability to inhibit adenylate cyclase activity, activate K<sup>+</sup> channels, and inhibit release of neurotransmitters via G<sub>i</sub><sup>1,2</sup>. Because the maximal effect of allodynia was observed 10–15 min after i.t. injection of PGF<sub>2α</sub> (Fig. 1), the effect might be mediated by other putative neurotransmitters. When baclofen was co-administered with PGF<sub>2α</sub>, it may presynaptically inhibit the release of neurotransmitters in the spinal cord. The blockade of the allodynia by baclofen was reversed by theophylline (Table 1), probably due to an increase in cAMP level by inhibition of phosphodiesterase. On the other hand, the effect of clonidine was antagonized by the α<sub>2</sub>–antagonist yohimbine<sup>9</sup>, but not by theophylline (Table 1). In the central nervous system, α<sub>2</sub>–adrenergic receptors are both presynaptic and postsynaptic<sup>8,14</sup>, and the mechanistic explanation for the inhibitory effect by clonidine on the allodynia seems not to be a simple one<sup>9</sup>. Although the precise mechanism of the PGF<sub>2α</sub>–induced allodynia is not known at present, the spinal α<sub>2</sub>–adrenergic and GABA<sub>B</sub> systems may exert a tonic inhibitory control on low threshold afferents. Recently, Hao et al.<sup>41</sup> observed allodynia in rats following laser–induced spinal cord injury. This allodynia was effectively relieved by i.p. injection of the GABA<sub>B</sub> agonist baclofen, but not by 24–h pretreatment with guanethidine. In this case, the GABA<sub>B</sub> system, but not the sympathetic system, is suggested to be involved in the laser–induced allodynia.

Allodynia is one of the biggest problems in pain management. The features of the PGF<sub>2α</sub>–induced allodynia apparently resemble those of patients suffering from reflex sympathetic dystrophy and causalgia. Our pharmacological studies revealed a prominent effect of drugs such as clonidine and baclofen, which are currently used clinically for managing chronic pain<sup>3,7</sup>, on the PGF<sub>2α</sub>–induced allodynia. To obtain further knowledge of the cellular mechanisms that trigger and maintain an increased excitability and to understand the long-term excitability control of the spinal cord receptors that signal pain, this allodynia may be a useful model for chronic pain.
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