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European Journal of Pharmacology 314 (1996) 313–317

ejp

Short communication

Study of paired-pulse inhibition of transcallosal response in the pyramidal tract neuron in vivo

Syed A. Chowdhury [a,*], Takashi Kawashima [a], Tokitaka Konishi [a], Masayuki Niwa [b], Ken'ichi Matsunami [a]

[a] *Department of Neurophysiology, Institute of Equilibrium Research, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500, Japan*
[b] *Department of Pharmacology, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500, Japan*

Received 17 June 1996; revised 14 August 1996; accepted 16 August 1996

Abstract

The effects of a specific $GABA_B$ receptor antagonist, *p*-(3-aminopropyl)-*p*-diethoxymethyl-phosphonic acid (CGP 35348), on pyramidal tract neuron responses to transcallosal stimulation were investigated in the cat motor cortex in vivo. The paired-pulse method was used to obtain more insight into the role of $GABA_B$ receptors. At a 200-ms inter-stimulus interval the spike response was inhibited in 75% of the neurons. There was an approximately 40% depression of the mean spike value in the control. CGP 35348 reduced paired-pulse inhibition, while (−)-baclofen increased it. Stronger drug effects on the second stimulation-induced response possibly indicate their presynaptic action on $GABA_B$ receptors.

Keywords: CGP 35348; (−)-Baclofen; Transcallosal response; Pyramidal tract neuron; Motor cortex; Paired-pulse method

1. Introduction

Recent investigations established the presence of two distinct classes of GABA receptors in the central nervous system: the $GABA_A$ and $GABA_B$ receptor (Alger and Nicoll, 1982; Bowery et al., 1990). Although the mechanism of action of the two receptor types differs, GABA (γ-aminobutyric acid) stimulation of both types of receptors results in neuronal inhibition. The function of the $GABA_A$ receptor has been well elucidated, but in contrast, $GABA_B$ receptor function is less well documented. Among several recently synthesized potent antagonists for the $GABA_B$ receptor, CGP 35348 (*p*-(-aminopropyl)-*p*-dihydroxymethyl-phosphonic acid), is one of the most highly specific $GABA_B$ receptor antagonist presently available (Olpe et al., 1990) whose chemical and pharmacological features have been well studied (Bittiger et al., 1990). To our knowledge most single cell studies with CGP 35348 were done either in slices or in cultured cells. Predicting the in vivo effects of a selective $GABA_B$ receptor antagonist within the central nervous system or even in the periphery is not easy, but in vivo experiments on $GABA_B$ function in the mammalian central nervous system are important to understand how $GABA_B$ receptors play their part in effects on neurons in the whole brain. Electrophysiological characteristics of callosal transmission have been well studied (Matsunami and Hamada, 1984). However, the pharmacological properties of the callosal synapse in cortical motor neurons in vivo have not been sufficiently investigated. In vivo experiments with motor cortical pyramidal tract neurons in relation to callosal GABA-ergic transmission are important, because (1) these are the most representative cortical output neurons and play an indispensable role in the execution of voluntary movements, (2) they receive rich dominant callosal inputs, (3) they cannot be identified in slice preparations, and (4) callosal transmission and GABA both are of clinical importance in epileptogenesis (Tunnicliff and Raess, 1991).

Previously we investigated the effect of CGP 35348 on transcallosal response of pyramidal tract neurons by using single stimulation (Chowdhury et al., 1995). In the present experiment we used the paired-pulse design to gain further insight into the $GABA_B$ receptor. The use of the paired-pulse paradigm is a well-established model to investigate the synaptic transmission system.

* Corresponding author. Tel.: (81-58) 267-2301; Fax: (81-58) 267-2954.

PII S0014-2999(96)00659-0

2. Materials and methods

Twenty-two adult cats of either sex were used in accordance with the NIH guidelines. Each cat was initially anesthetized with ketamine hydrochloride (10 mg/kg i.m.). Tracheotomy was performed for artificial ventilation. A silicon tube was inserted in the right cephalic vein, in order to maintain anesthesia throughout the experiments with additional doses of diluted ketamine hydrochloride (2 mg/kg per hour, i.v.). Cut edges were infiltrated with local anesthetics. A stimulation electrode consisting of eight acupuncture needles was inserted in the left motor cortex.

Another stimulation electrode was set in the pyramidal tract by the dorsal approach after a small hole was made in the occipital bone to identify the pyramidal tract neurons. Pyramidal tract neurons were identified by conventional criteria. Recordings were made from the forepaw region of the right motor cortex. A multi-barrel carbon fiber glass microelectrode, its center barrel filled with 2 M NaCl, was used for single unit recording. The peripheral barrels were filled with CGP 35348 (10 mM) and (−)-baclofen (20 mM). Paired-pulse stimuli were delivered at an interval of 200 ms. The main interval was fixed at 2 s. The strength of the stimulation was kept near the threshold level of the firing to obtain a clear drug effect. Neuronal activity was sampled for 20 trials of transcallosal stimulation, with a signal processor (7T17, NEC-Sanei). Pyramidal tract neuron activity and rastergrams were displayed on the screen of the signal processor, and on chart sheets. The data were stored on floppy disk. After control data were obtained, application of drugs was started. First, CGP 35348 was applied by iontophoresis (20 nA positive current, 1 min). Soon after completion of CGP 35348 application, paired-pulse stimuli were delivered to drive neuronal activity. After completion of recording, (−)-baclofen was applied by positive iontophoretic current (40 nA, 1 min) to examine the effect of (−)-baclofen on CGP 35348 action. After the experiments were over, off-line analysis was conducted. The spikes in 30 ms from the start of the stimulus for 20 pairs of transcallosal stimulations were counted on the rastergrams re-drawn on chart sheets (30.5 × 22.0 cm). The latency of neuronal activity was measured as the latency of the earliest spike incidence. An F-test was used

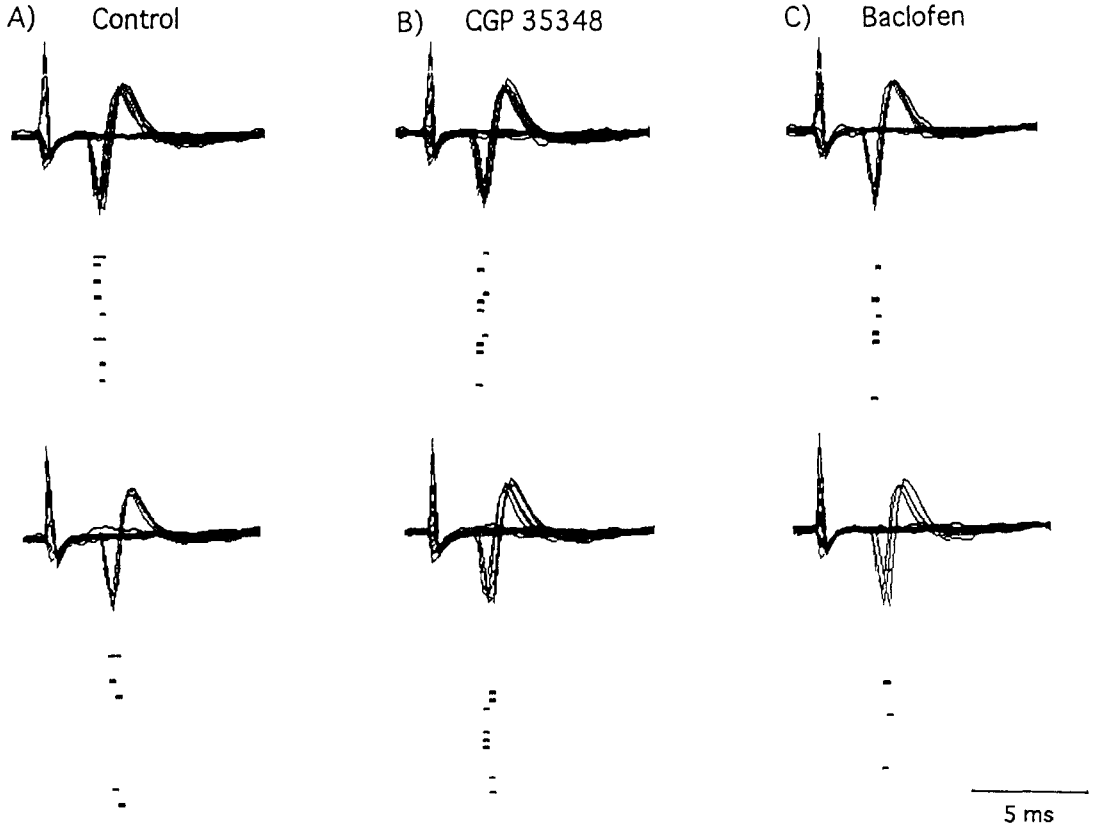

Fig. 1. A specimen record of neuronal activity. Each frame shows 20 superimposed traces. Upper superimposed traces of A, B and C are first stimulation-induced responses and lower traces are second stimulation-induced responses. Raster display is shown in the lower part of each frame. (A) Paired-pulse inhibition of the spike response in the control. (B) Paired-pulse inhibition was reversed by iontophoretic application of CGP 35348, and (−)-baclofen again increased the paired-pulse inhibition, as shown in panel C.

for statistical analysis when the difference in covariances between the two groups was large. Otherwise the paired t-test was used.

3. Results

Single unit activity of a total of 32 pyramidal tract neurons was investigated using the paired-pulse method. When two consecutive stimuli were applied at an inter-stimulus interval of 200 ms, the response of spikes was reduced by the second stimulation. In pilot experiments, we observed that the degree of inhibition depends on the duration of the stimulation interval. Maximum inhibition was observed at an inter-stimulus interval of 200 ms. At this interval, inhibition of the spike response was observed in 75% of the neurons (24 of 32). Paired-pulse facilitation was observed in 16% of the neurons (5 out of 32). Nine percent of the neurons (3 out of 32) did not show any changes with the second stimulation. Iontophoretic application of CGP 35348 (10 mM, 1 min) reduced the paired-pulse inhibition greatly but not completely in most instances. Forty percent depression of the mean spike value was observed in the control. The value decreased to 20.6% by CGP 35348 and was increased to 34% by (−)-baclofen.

Fig. 1 provides an example of paired-pulse depression of the spike response and the effect of drugs on the transcallosal response of pyramidal tract neurons. Twenty traces of original potentials are superposed and demonstrated in the upper half of the figure; their rastergrams are displayed in the lower half of each frame. Fig. 1A illustrates the control transcallosal response. Eight spikes were activated by 20 paired-pulse stimulations. At a 200-ms inter-stimulus interval, paired-pulse depression was observed and only 5 spikes were activated by 20 stimulations. When CGP 35348 was applied, paired-pulse depression was reduced and the number of spikes increased to 8. CGP 35348 also increased the first stimulation-induced spike response from 8 to 9 (Fig. 1B). A selective $GABA_B$ receptor agonist, (−)-baclofen, had an effect opposite to that of CGP 35348 on paired-pulse depression (Fig. 1C). Note that complete reduction of paired-pulse depression was only observed in a few instances by CGP 35348.

Table 1 shows the changes of mean spike values and mean latencies by drugs. Drug effects on both first and second stimulation-induced responses are given. In the case of spike changes, the effect of CGP 35348 was greater on the paired-pulse inhibition ($P < 0.001$) than on the first stimulation-induced response ($P < 0.05$). As the first stimulation-induced population spikes were also reduced by (−)-baclofen ($P < 0.05$) the paired-pulse inhibition was mainly increased by the greater inhibition of the second stimulation-induced spikes ($P < 0.001$).

It must be noted, however, that statistical analysis was only applied to those units that showed paired-pulse depression. Six units were discarded during the analysis of latency because in the course of the experiments the spikes were somehow completely eliminated by the drugs. CGP 35348 shortened the second stimulation-induced response latency significantly but baclofen lengthened the response latency in both first and second stimulation-induced responses non-significantly (t-test, $P > 0.05$; exact P values were $P = 0.13$ and 0.056, respectively). Therefore, the results of our present experiments suggest that, although CGP 35348 and baclofen had an effect on both first and second stimulation-induced response, the effects of the drugs were stronger on the paired-pulse-induced depression.

Table 1
Mean values for the spike number and latency are shown in both first and second stimulation-induced responses

	Control	CGP 35348	Baclofen
Mean spike ± S.D.			
1st stimulation	10.5 ± 8.9	13.6 ± 12.8 [b]	10.6 ± 12.2 [b]
2nd stimulation	6.3 ± 6.5	10.8 ± 9.5 [a]	7.0 ± 8.2 [a]
Mean latency ± S.D.			
1st stimulation	3.4 ± 1.8	3.2 ± 1.9	3.9 ± 3.0
2nd stimulation	4.7 ± 3.4	3.4 ± 2.8 [b]	5.1 ± 4.2

Drug effects were stronger on the second stimulation-induced responses. Significance: [a] $P < 0.001$ and [b] $P < 0.05$, effects of CGP 35348 and (−)-baclofen were compared to control and CGP 35348, respectively (paired t-test).

4. Discussion

The strength of synaptic transmission is not fixed, but can increase or decrease as a result of previous activity (Zucker, 1989). Frequency-dependent facilitation and depression have been observed at several synapses (Kuno, 1964; Deisz and Prince, 1989; Mott et al., 1993). A possible role of GABA, a central inhibitory neurotransmitter, in regulating the strength of synaptic transmission has been hypothesized. Such studies have been facilitated by the development of potent and selective $GABA_B$ receptor antagonists and of techniques for studying monosynaptic inhibitory responses. We now examined the paired-pulse inhibition of the spike response of pyramidal tract neurons induced by transcallosal stimulation at an identical inter-stimulus interval under conditions where all receptor systems were apparently activated, or remained intact or functional.

At most inhibitory synapses of the brain, GABA activates two quantitatively different inhibitory mechanisms through $GABA_A$ and $GABA_B$ receptors. $GABA_B$ receptors can be located both pre- and postsynaptically. The postsynaptic mechanisms are well understood and rela-

tively simple. The presynaptic mechanisms, however, are not nearly so well understood because intracellular recording from most nerve terminals is not easy and the mechanisms proposed were based on the extrapolation of findings for the parent cell bodies. However, several previous studies have provided evidence for $GABA_B$ receptors in presynaptic afferent terminals, which modify transmitter release by presynaptic inhibition from excitatory (Dutar and Nicoll, 1988; Edward et al., 1989) as well as inhibitory terminals. Results of many experiments have shown that paired-pulse depression of the inhibitory postsynaptic potential is mediated by the inactivation of $GABA_B$ receptors at the inhibitory terminals. There have been only a few studies that have demonstrated the paired-pulse depression of excitatory postsynaptic events (Forsythe and Clements, 1990; Kang, 1995) and it was reported that possibly the presynaptic but not the postsynaptic $GABA_B$ receptor is involved in this process (Huang and Gean, 1994). These findings of a frequency-dependent inhibition of excitatory postsynaptic events in slices are in line with the data obtained from our in vivo experiments where paired-pulse inhibition of neuronal excitability was observed and in which iontophoretically applied CGP 35348 reduced the inhibition and (−)-baclofen increased it. It is generally accepted that stimulation-induced action potentials usually generate in the excitatory postsynaptic potentials. CGP 35348 has been reported as a selective blocker of presynaptic $GABA_B$ receptors (Davies et al., 1991). On the other hand, the effect of baclofen on presynaptic terminals has been documented. It was observed that baclofen suppresses the release of a variety of transmitters (Bowery et al., 1980). In low concentrations, baclofen selectively inhibits the release of excitatory amino acids from cortical slices (Potashner, 1979). We cannot rule out the possibility of a spike response in the early inhibitory potential, but even if this happened it is likely that the number of action potentials should be increased at the second stimulation, in most instances at least.

Therefore, our results suggest that the inhibition of the spike response by the second stimulation of the paired-pulse is possibly due to the depression of the excitatory potential, and that presynaptic $GABA_B$ receptors regulate the stimulation-induced excitability of the motor cortical pyramidal tract neurons.

In conclusion, it is not clear why, in most instances, a potent $GABA_B$ antagonist failed to block the paired-pulse inhibition completely. The lack of a CGP 35348 effect can be explained on the basis of previous experiments: firstly, $GABA_B$-independent paired-pulse depression has been reported (Lambert and Wilson, 1994). Both muscarinic and $GABA_B$-ergic paired-pulse inhibition of the *N*-methyl-D-aspartate (NMDA)-sensitive excitatory postsynaptic current (EPSC) has been proposed recently (Kang, 1995). Secondly, continuous activation of the presynaptic $GABA_B$ receptors by endogenous GABA could be another possibility.

Acknowledgements

We are greatly indebted to Ciba-Geigy Ltd. for the kind gift of CGP 35348. We also express our thanks to Miss Y. Nishijima for the illustrations. This research was supported by a grant from the Japanese Ministry of Education, Science and Culture.

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