ELSEVIER

European Journal of Pharmacology 285 (1995) 99–102

ejp

Short communication

$GABA_B$ receptor antagonist CGP 35348 shortens transcallosal response latency of pyramidal tract neurons

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

[a] *Institute of Equilibrium Research, Gifu University School of Medicine, Tsukasamachi 40, Gifu 500, Japan*
[b] *Department of Pharmacology, Gifu University School of Medicine, Tsukasamachi 40, Gifu 500, Japan*

Received 27 April 1995; revised 1 August 1995; accepted 4 August 1995

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. Iontophoretic application of CGP 35348 significantly increased the number of spikes from 10.3 ± 4.4 (control; $n = 27$; mean ± S.D.) to 16.7 ± 7.2 (CGP 35348) for 20 transcallosal stimulation trials, while the latency of neuronal activity was significantly shortened from 4.4 ± 2.1 ms (control; $n = 27$; mean ± S.D.) to 3.8 ± 1.7 ms (CGP 35348). In conclusion, CGP 35348 facilitated transcallosal synaptic transmission between pyramidal tract neurons by removal of $GABA_B$ inhibition.

Keywords: $GABA_B$ receptor antagonist; CGP 35348; Pyramidal tract neuron; Transcallosal response, cat; Motor cortex

1. Introduction

Recent investigations established the presence of two subtypes of GABA (γ-aminobutyric acid) receptors in the central nervous system, $GABA_A$ and $GABA_B$ receptors. The function of $GABA_A$ receptors has been well elucidated. The $GABA_B$ receptor has recently been categorized as another subtype of the GABA receptor and causes slow inhibitory postsynaptic potentials (IPSPs: Bowery et al., 1980, 1990; Dutar and Nicoll, 1988). Several $GABA_B$ receptor antagonists have been recently synthesized (Bowery et al., 1990; Kerr et al., 1990), including CGP 35348 (*p*-(3-aminopropyl)-*p*-diethoxymethyl-phosphonic acid) which is one of the most highly specific $GABA_B$ receptor antagonists presently available (Olpe et al., 1990) whose chemical and pharmacological features have been well studied (Bittiger et al., 1990). However, their pharmacological effects on cortical neurons in vivo, particularly on pyramidal tract neurons, have not been sufficiently investigated. In vivo experiments with pyramidal tract neurons are important, because (1) they are the most representative cortical output neurons and play an indispensable role in the execution of voluntary movements (Evarts, 1965), (2) they cannot be identified in slice preparations, and (3) they receive rich dominant callosal inputs, whose electrophysiological nature in motor control has been well studied (Matsunami and Hamada, 1984), and have clinical importance in epileptogenesis (Reeves, 1985). For these reasons, we tested the effects of a specific $GABA_B$ receptor antagonist, CGP 35348, on the transcallosal response of pyramidal tract neurons in an in vivo preparation.

2. Materials and methods

Sixteen cats were used in accordance with the NIH guidelines. Each cat was anesthetized with ketamine chloride (10 mg/kg i.m.) and artificially ventilated. A silicon tube was inserted in the cephalic vein, in order to maintain the anesthetic conditions throughout the experiments by additional doses of diluted ketamine chloride. The skull was opened over the pericruciate regions on both sides. A stimulating electrode consisting of eight acupuncture needles was inserted in the

* Corresponding author. Tel.: 058-265-1241; fax: 058-267-2954.

SSDI 0014-2999(95)00483-1

left motor cortex. Recordings were made from the forepaw region of the right motor cortex. Another stimulation electrode was set in the pyramidal tract to identify the pyramidal tract neuron. Pyramidal tract neuron activity was identified by conventional criteria.

A multi-barrel glass pipette electrode, its center barrel filled with fine carbon fiber (7 μm in diameter; 5–7 MΩ) and 2 M NaCl, was used for unit recording (Sawaguchi et al., 1986). The peripheral barrels were filled with CGP 35348 (10 mM) and GABA (10 mM). The amount of iontophoretic current was positive and 20 nA for 1 min for both drugs.

Neuronal activity was sampled for 20 trials of transcallosal stimulation, by sampling at a rate of 0.2 ms/bin to make peri-stimulus rastergrams with a signal processor (7T17, NEC-Sanei). Thirty milliseconds was allotted for a pre-stimulus period and 70 ms for a post-stimulus period. Pyramidal tract neuron activity and rastergrams were displayed on the screen (12 inches) of the signal processor, and on chart sheets. The data were stored on floppy disk. After control data were obtained, application of drugs was begun. First, CGP

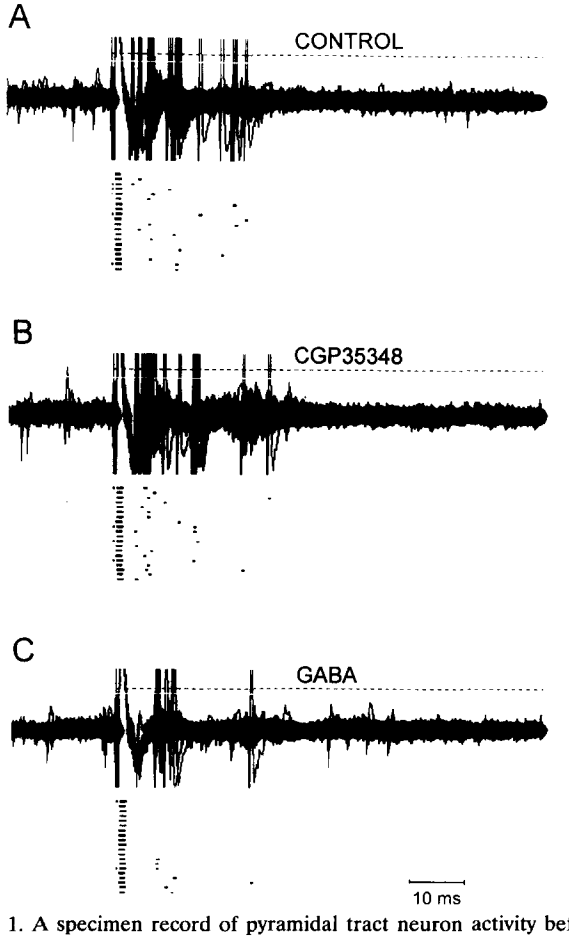

Fig. 1. A specimen record of pyramidal tract neuron activity before iontophoretic application of CGP 35348 (A, control), after CGP 35348 application (B) and GABA application (C). Twenty original traces are superposed in the upper half of each frame. Their rastergrams are illustrated in the lower half. Horizontal bar is 10 ms.

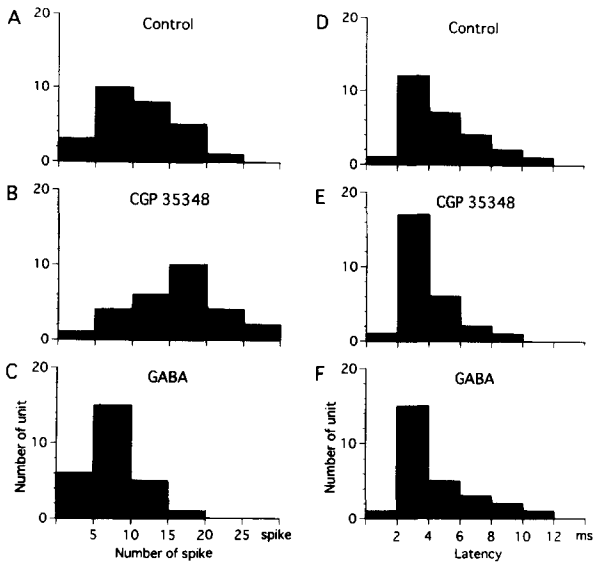

Fig. 2. Distribution histogram showing number of spikes and latency of individual pyramidal tract neurons. A, a histogram of control. B, a histogram for CGP 35348 application. C, a histogram for GABA application. Latency histograms for control (D), after CGP 35348 (E), and GABA application (F). Spikes for 20 transcallosal stimulations were summed for each pyramidal tract neuron to obtain the spike number.

35348 was applied. Soon after completion of CGP 35348 application, transcallosal stimulation was delivered to drive neuronal activity. After 5 min, when the effects of 35348 CGP had almost subsided, GABA was applied. Peri-stimulus rastergrams were similarly made.

After the experiments were over, the off-line analysis was conducted. The number of spikes in 30 ms from the stimulus onset for 20 transcallosal stimulations were counted on the rastergrams re-drawn on chart sheets (30.5 × 22.0 cm). A duration of 30 ms was determined on the basis of the fact that the early two transcallosal potentials (P1 and N1 waves) almost ended within this period. The latency of neuronal activity was measured as the latency of the earliest spike incidence. The *F*-test was used for statistical analysis when a difference of covariances between the two groups was large. Otherwise the paired *t*-test was used.

3. Results

Fig. 1 provides an example of pyramidal tract neuron activity after transcallosal stimulation. Twenty traces of original potentials are superposed and demonstrated in the upper half; their rastergrams are displayed in the lower half of each frame (Fig. 1A, B and C). Fig. 1A illustrates the control transcallosal response. Eighteen spikes were activated for 20 transcallosal stimulation trials. When CGP 35348 was applied, the number of spikes increased to 22 (Fig. 1B).

When GABA was applied, the number of spikes decreased to seven (Fig. 1C).

Fig. 2 gives distribution histograms of the numbers of spikes and the latencies of 27 pyramidal tract neurons for the control, and after CGP 35348 and GABA application. The mean number of spikes in response to transcallosal stimulation for the control was 10.3 ± 4.4 ($n = 27$; mean $\pm$ S.D.; Fig. 2A). The mean latency was 4.4 ± 2.1 ms ($n = 27$; mean $\pm$ S.D.; Fig. 2D). When CGP 35348 was applied, the mean spike number significantly increased to 16.7 ± 7.2 (t-test, $P < 0.001$). It must be noted, however, that one pyramidal tract neuron showed a decreasing trend in the number of spikes. This neuron was included in the illustration of the histogram when calculating the mean spike number. The mean latency significantly decreased to 3.8 ± 1.7 ms ($n = 27$; t-test, $P < 0.01$). When GABA was applied, the mean spike number significantly decreased to 7.8 ± 3.7 ($n = 27$) from that of CGP 35348 application (F-test, $P < 0.01$), and even from the control value (t-test, $P < 0.01$). The mean latency ($n = 27$; 4.3 ± 2.2 ms) returned almost to the control value.

4. Discussion

The present experiments demonstrated in the cat motor cortex that the $GABA_B$ receptor antagonist CGP 35348 increased the number of spikes and shortened the latency of pyramidal tract neuron activity in response to transcallosal stimulation. These changes were in the same direction as expected from the pharmacological nature of CGP 35348 (Olpe et al., 1990).

When intracellular recordings were made in cortical neurons in slice preparation, fast and slow IPSPs were observed in response to callosal stimulation (Vogt and Gorman, 1982; Kawaguchi, 1992). Fast IPSPs were accompanied by an increase in $GABA_A$-mediated Cl^- conductance and were blocked by bicuculline (Kawaguchi, 1992). $GABA_B$ receptor stimulation produced late IPSPs with slower temporal development of K^+ conductance (Dutar and Nicoll, 1988; Kawaguchi, 1992). CGP 35348 is a highly specific and potent new $GABA_B$ receptor blocker (Bittiger et al., 1990; Olpe et al., 1990). The increase of spike number and the decrease of latency in response to transcallosal stimulation can be explained by the antagonistic action of CGP 35348 on $GABA_B$ receptors. The first explanation for the present results may involve presynaptic events. The $GABA_B$ agonist baclofen reduced excitatory postsynaptic potentials (EPSPs) by reducing transmitter release from the presynaptic terminals by way of presynaptic $GABA_B$ receptors (Edwards et al., 1989; Thompson and Gahwiler, 1992). Application of CGP 35348 may remove this presynaptic inhibition to thus facilitate the EPSP response to transcallosal stimulation. The second possibility is a reduction of postsynaptic $GABA_B$ IPSP by CGP 35348, although effects may be small in the early phase of the transcallosal response because of the slower developmental time course of the $GABA_B$ IPSP. Thirdly, the existence of $GABA_B$ tone is suggested (Olpe et al., 1992). Removal of a $GABA_B$ inhibitory tone may be another way to facilitate excitability under transcallosal stimulation. In conclusion, CGP 35348 facilitated transcallosal synaptic transmission by removal of $GABA_B$ inhibition, most presumably by a presynaptic action.

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.

References

Bittiger, H., W. Froestl, R. Hall, G. Karlsson, K. Klebs, H.-R. Olpe, M.F. Pozza, M.V. Steinmann and H. Van Riezen, 1990, Biochemistry, electrophysiology and pharmacology of a new $GABA_B$ antagonist: CGP 35348, in: $GABA_B$ Receptors in Mammalian Function, eds. N.G. Bowery, H. Bittiger and H.-R. Olpe (John Wiley and Sons, Chichester) p. 47.

Bowery, N.G., D.R. Hill, A.L. Hudson, A. Doble, D.N. Middlemiss, J. Shaw and M. Turnbull, 1980, (−) Baclofen decrease neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor, Nature 283, 92.

Bowery, N.G., H. Bittiger and H.-R. Olpe (eds.), 1990, $GABA_B$ Receptors in Mammalian Function (John Wiley and Sons, Chichester) p. 442.

Dutar, P. and R.A. Nicoll, 1988, Physiological role for GABA B receptors in the central nervous system, Nature 332, 156.

Edwards, F.R., P.J. Harrison, J.J.B. Jack and D.M. Kullmann, 1989, Reduction by baclofen of monosynaptic EPSPs in lumbosacral motoneurones of the anaesthetized cat, J. Physiol. (London) 416, 539.

Evarts, E.V., 1965, Pyramidal tract activity associated with a conditioned hand movement in the monkey, J. Neurophysiol. 29, 1011.

Kawaguchi, Y., 1992, Receptor subtypes involved in callosally induced postsynaptic potentials in rat frontal agranular cortex in vitro, Exp. Brain Res. 88, 33.

Kerr, D.I.B., J. Ong and R.H. Prager, 1990, $GABA_B$ receptor agonists and antagonists. in: $GABA_B$ Receptors in Mammalian Function, eds. N.G. Bowery, H. Bittiger and H.-R. Olpe (John Wiley and Sons, Chichester) p. 27.

Matsunami, K. and I. Hamada, 1984, Effects of stimulation of corpus callosum on precentral neuron activity in the awake monkey, J. Neurophysiol. 52, 676.

Olpe, H.-R., G. Karlsson, M.F. Pozza, M. Steinmann, H. Van Riezen, G. Fagg, W. Froestl and H. Bittiger, 1990, CGP 35348: a centrally active blocker of $GABA_B$ receptors, Eur. J. Pharmacol. 187, 27.

Olpe, H.-R., T.H. Ferrat, W. Worner, P. Andre and M.W. Steinmann, 1992, Evidence for a '$GABA_B$ tone' in vivo: the effect of CGP35348 on long-term potentiation, paired-pulse inhibition and cortical excitability, Pharmacol. Commun. 2, 124.

Reeves, A.G. (ed.), 1985, Epilepsy and the Corpus Callosum (Plenum Press, New York) p. 534.

Sawaguchi, T., M. Matsumura and K. Kubota, 1986, Long-lasting marks of extracellularly recorded sites by carbon fiber glass-micropipettes in the frontal cortex of chronic monkeys, J. Neurosci. Methods 15, 341.

Thompson, S.M. and B.H. Gahwiler, 1992, Comparison of the actions of baclofen at pre- and postsynaptic receptors in the rat hippocampus in vitro, J. Physiol. (London) 451, 329.

Vogt, B.A. and A.L.F. Gorman, 1982, Response of cortical neurons to stimulation of corpus callosum in vitro, J. Neurophysiol. 48, 1257.