Late inhibitory postsynaptic potentials in rat prefrontal cortex may be mediated by $GABA_B$ receptors

G. Karlsson and H.-R. Olpe*

Biology Research Department, Pharmaceuticals Division, Ciba-Geigy Ltd, CH-4002 Basle (Switzerland)
Received 26 July 1988; accepted 19 October 1988

Summary. The $GABA_B$ antagonist phaclofen blocked the postsynaptic hyperpolarization induced by the $GABA_B$ agonist baclofen during intracellular recordings in rat cortical cells. This effect appears to be selective since responses to $GABA_A$ agonists (muscimol, THIP), GABA, 5-HT and L-glutamate were unaffected. Phaclofen also blocked synaptically evoked late inhibitory postsynaptic potentials (late IPSP). These results suggest that the late IPSPs in cortical neurons are mediated by GABA acting on $GABA_B$ receptors.
Key words. Phaclofen; late inhibitory postsynaptic potential; cortex; GABA; baclofen.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. It mediates its action via two classes of receptors, $GABA_A$ and $GABA_B$[1,2]. Whereas the physiology of $GABA_A$ receptors is understood in considerable detail, the role of $GABA_B$ receptors has been less thoroughly investigated. Activation of $GABA_B$ receptors has been shown to inhibit the release of various transmitters centrally[3,4] and peripherally[5]. More recently, it was demonstrated that $GABA_B$ receptors mediate the late inhibitory postsynaptic potential (IPSP) in the hippocampus, thalamus and septum[6-8]. This late IPSP reverses near the equilibrium potential for potassium (K^+)[9] and resembles the K^+-dependent hyperpolarization induced by the $GABA_B$ agonist baclofen[10,11]. In the present study, we have investigated whether the late IPSP recorded in cortical neurons is also mediated via $GABA_B$ receptors. The selectivity of the new $GABA_B$ blocker phaclofen[12] has not been extensively tested[6], and to date, no data on phaclofen binding has been reported. Therefore, in one series of experiments, the potency and selectivity of the new $GABA_B$ blocker phaclofen in cortical tissue was determined. Subsequently, the effect of phaclofen on the late IPSP in the cortex was investigated. Some of these results have appeared in a preliminary report[13].

Methods. Male Sprague-Dawley rats (80–150 g) (RA 25, Tif, Sisseln, Switzerland) were decapitated under light ether anesthesia. The brain was rapidly removed and immersed for 10 s in a cooled artificial cerebrospinal fluid (ACSF) solution (3–5 °C) pregassed with 95% O_2/5% CO_2. Transverse slices (500 µm) of the medial frontal cortex were cut with a Vibratome. Slices were maintained semi-submerged in a modified version of the Haas chamber[14] and perfused, the first hour at room temperature and then at 33–35 °C, in ACSF (pH 7.4) of the following composition (mM): NaCl 124, KCl 2.5, $CaCl_2$ 2.5, $MgSO_4$ 2, KH_2PO_4 1.25, $NaHCO_3$ 26 and D-glucose 10. Intracellular recordings were made from cortical neurons in the cell layers 2–4 with glass microelectrodes filled with 2 M potassium methylsulphate (resistance 70–120 MΩ). An Axoclamp-2A amplifier was used. Changes in input resistance were determined by injecting constant current hyperpolarizing pulses every 60 s through the recording electrode. Postsynaptic potentials were evoked by constant current stimulation (60–140 µA; 0.6–2.5-ms duration) (Grass S11 stimulator with stimulus isolation unit PSIU6) of superficial cortical layers through a bipolar stainless steel electrode. The following drugs were used: baclofen (Ciba-Geigy, Basle), phaclofen (Dr R. G. Hall, Ciba-Geigy, Manchester, UK and Tocris Neuramin), muscimol (Ciba-Geigy, Basle), THIP (Research Biochemicals Inc.), GABA (Serva), 5-HT (Fluka) and L-glutamate (Fluka). All drugs were dissolved in ACSF and added to the perfusion chamber via the main perfusion line.

Results. Recordings were obtained from 45 cortical neurons which had resting membrane potentials more negative than −50 mV (−68.8+/−1.5 mV; mean+/−SEM). Phaclofen (0.5–1 mM) had no consistent effect on input resistance or resting membrane potential in cortical cells (n = 26). At the highest concentration (1 mM), however, phaclofen induced a weak transient depolarization (1–2 mV) of the membrane potential in 4 out of 8 cells tested. Baclofen at 1–10 µM produced a hyperpolarization which was associated with a mild decrease in membrane input resistance (data not shown). At 1 mM, phaclofen inhibited the maximal response to baclofen (10 µM) by 72.6+/−7.7% (mean+/−SEM) (n = 9). The inhibition occurred rapidly (4–6 min) and recovered within 10–12 min from the start of the washout. The effect of 0.5 mM phaclofen on baclofen-induced (1–10 µM) responses (n = 4, data not shown) was not as consistent and reproducible as the results obtained with 1 mM phaclofen. We therefore used 1 mM phaclofen in the remaining experiments of this series. The $GABA_A$ agonists muscimol (3 µM, n = 2) and THIP (0.5 mM, n = 2) both induced membrane depolarization. Phaclofen at 1 mM had no effect on responses to either agonist (data not shown). GABA (0.5–1 mM, n = 4) produced membrane depolarization, which in two cells was followed by a brief hyperpolarization. On the other hand, 5-HT (0.2 mM, n = 2), hyperpolarized the cells. However, phaclofen had no effect on responses to either GABA or 5-HT in the same cell (data not shown). The depolarizing response induced by L-glutamate (0.5 mM, n = 4) always resulted in cell firing. The amplitude of the L-glutamate-induced response was slightly reduced during phaclofen-perfusion (1 mM). Figure 1 depicts an example of the effect of phaclofen on baclofen- and L-glutamate-induced responses in the same cell.

Electrical stimulation of superficial layers of the cortical slice evoked a biphasic IPSP in 19 out of 29 neurons impaled (fig. 2). The remaining cells (n = 10) showed an action potential riding on a pure depolarizing potential (data not shown). In all neurons tested, phaclofen (0.1–1 mM) reversibly reduced the stimulation-evoked late IPSP. The onset of action was 3–4 min at 0.1 mM (n = 4), 2–3 min at 0.5 mM (n = 7) and 1–2 min at 1 mM (n = 2). Recovery

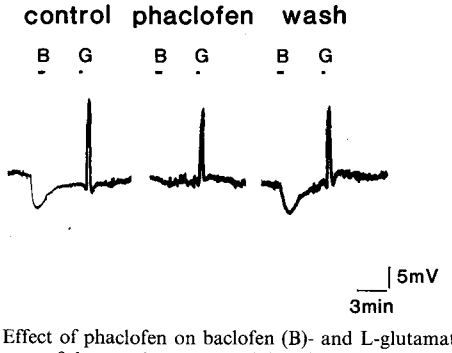

Figure 1. Effect of phaclofen on baclofen (B)- and L-glutamate (G)-induced changes of the membrane potential during intracellular recording in rat cortical neurons. Phaclofen (1 mM) was perfused 5 min before as well as during the baclofen (10 µM, 45 s) and L-glutamate (0.5 mM, 20 s) perfusions. Resting membrane potential of the cell was −82 mV.

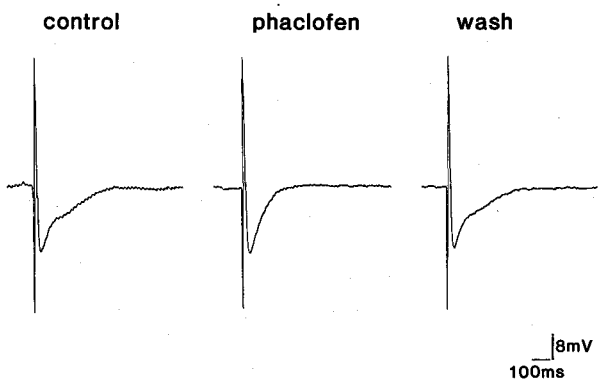

Figure 2. Effect of phaclofen (1 mM, 4 min) on synaptically evoked late IPSP during intracellular recording in rat cortical neurons. Resting membrane potential of the cell was −63 mV.

occurred 4–8 min and 8–12 min after start of the washout of the low (0.1 mM) and higher (0.5–1 mM) doses, respectively. Phaclofen up to 1 mM had no effect on the synaptically elicited EPSP, the action potential or the early IPSP (data not shown). Figure 2 shows a typical example in which phaclofen depresses the late IPSP in a reversible manner.

Discussion. In the present study we demonstrate that phaclofen, although originally claimed to be a weak presynaptic $GABA_B$ antagonist on spinal neurons [12], is a remarkably selective blocker of the postsynaptic action of baclofen, and that it selectively abolishes the late IPSP in cortical cells. The fact that phaclofen inhibited the K^+-dependent [10,11] $GABA_B$-mediated action of baclofen, but had no effect on responses induced by the $GABA_A$ agonists, muscimol and THIP, strongly indicates that the effect of phaclofen is selective on $GABA_B$ receptors. Moreover, phaclofen did not affect the action of 5-HT, which has been shown to be mediated by the same K^+ channels but not by the same receptors as baclofen-induced responses [15]. This is in agreement with what has been reported in the hippocampus [6]. At moderate concentrations phaclofen had no effect on basic membrane properties. At higher concentrations (1 mM), it induced a weak transient depolarization. This might be due to a reduction in tonic input of GABA. In the presence of phaclofen, the effect of glutamate appeared to be slightly reduced (fig. 1). However, this effect may be indirect, arising from the tendency towards membrane depolarization during phaclofen-perfusion. The observation that phaclofen markedly reduced baclofen-induced responses but had no effect on those of GABA may seem surprising. However, in our experiments GABA primarily elicited depolarizing responses. According to findings by Scharfman and Sarvey [16] this effect is due to dendritic GABA receptor activation. Furthermore, it has been shown in the hippocampus that dendritic GABA responses mediated by $GABA_A$ receptors predominate, and mask responses mediated by $GABA_B$ receptors [17].

The effect of phaclofen on synaptically evoked postsynaptic potentials in the present study supports recent findings in other brain areas [6–8] that phaclofen selectively inhibits the $GABA_B$-mediated late IPSP, but not the EPSP and the $GABA_A$-mediated early IPSP. The involvement of the $GABA_B$ receptor in the induction of the late IPSP in the cortex is further supported by the findings that the $GABA_B$ receptor is coupled to a K^+ channel and that the late IPSP is sensitive to changes in external K^+ concentrations [9]. However, relatively high stimulation intensities are needed to elicit the late IPSP (own observations [6,9]). This suggests that the activation of the $GABA_B$ receptor depends on the strength of the afferent input, i.e. on the amount of GABA released. This concurs with studies showing that higher concentrations of GABA are required for the activation of $GABA_B$ than of $GABA_A$ receptors [17]. Phaclofen, which has been reported to be a weak competitive antagonist of baclofen [7], was effective in reducing the late IPSP at much lower concentrations than those needed to antagonize baclofen-induced responses. Thus it seems that phaclofen in this study was potent enough to compete with endogenously released GABA for the $GABA_B$ receptor and was therefore able to block the synaptically evoked late IPSP.

In summary, we have demonstrated for the first time that phaclofen, the only available $GABA_B$-blocker, is a useful pharmacological tool which selectively antagonizes baclofen-induced hyperpolarizations recorded from rat cortical neurons. Furthermore, phaclofen blocks the late IPSP, thus providing strong evidence for the involvement of $GABA_B$ receptors in synaptic transmission. The presence of late IPSPs [6–8] as well as $GABA_B$ binding [2] in various brain areas suggest that this $GABA_B$ receptor-mediated IPSP represents a relatively common inhibitory mechanism in the mammalian CNS.

* To whom reprint requests should be addressed.

1 Alger, B. E., and Nicoll, R. A., J. Physiol. *328* (1982) 125.
2 Bowery, N. G., Hudson, A. L., and Price, D. A., Neuroscience *20* (1987) 365.
3 Bowery, N. G., Hill, D. R., Hudson, A. L., Doble, A., Middlemiss, D. N., Shaw, J. S., and Turnbull, M. J., Nature *283* (1980) 92.
4 Olpe, H. R., Baudry, M., Fagni, L., and Lynch, G., J. Neurosci. *2* (1982) 698.
5 Bowery, N. G., Doble, A., Hill, D. R., Hudson, A. L., Shaw, J. S., Turnbull, M. J., and Warrington, R., Eur. J. Pharmac. *71* (1981) 53.
6 Dutar, P., and Nicoll, R. A., Nature *332* (1988) 156.
7 Hasuo, H., and Gallagher, J. P., Neurosci. Lett. *86* (1988) 77.
8 Soltesz, I., Haby, M., Leresche, N., and Crunelli, V., Brain Res. *448* (1988) 351.
9 Howe, J. R., Sutor, B., and Zieglgänsberger, W., Cell. molec. Neurobiol. *7* (1987) 1.
10 Gähwiler, B. H., and Brown, D. A., Proc. natl Acad. Sci. USA *82* (1985) 1558.
11 Howe, J. R., Sutor, B., and Zieglgänsberger, W., J. Physiol. (Lond.) *384* (1987) 539.
12 Kerr, D. I. B., Ong, J., Prager, R. H., Gynther, B. D., and Curtis, D. R., Brain Res. *405* (1987) 150.
13 Karlsson, G., Pozza, M., and Olpe, H. R., Eur. J. Pharmac. *148* (1988) 485.
14 Haas, H. L., Schärer, B., and Vosmansky, M., J. Neurosci. Meth. *1* (1979) 323.
15 Andrade, R., Malenka, R. C., and Nicoll, R. A., Science *234* (1986) 1261.
16 Scharfman, H. E., and Sarvey, J. M., Brain Res. *358* (1985) 385.
17 Newberry, N. R., and Nicoll, R. A., J. Physiol. (Lond.) *360* (1985) 161.

0014-4754/89/020157-02$1.50 + 0.20/0