Is β-(4-Chlorophenyl)-GABA a Specific Antagonist of Substance P on Cerebral Cortical Neurons?

J. W. PHILLIS[1]

Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon S7N OWO (Saskatchewan, Canada), 10 December 1975.

Summary. β-(4-chlorophenyl)-GABA (Baclofen, Lioresal) antagonized the excitant actions of acetylcholine and substance P to comparable extents. L-glutamate-induced excitation was affected to a lesser extent These findings do not support the suggestion that β-(4-chlorophenyl)-GABA is a specific substance P antagonist.

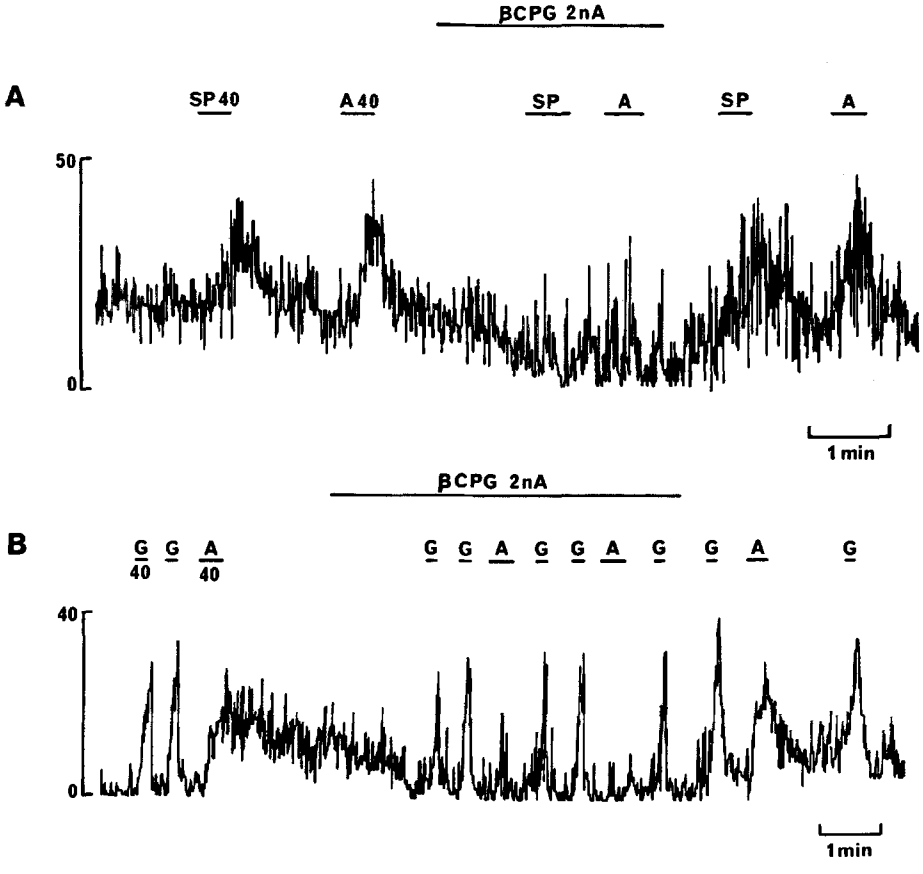
β-(4-Chlorophenyl)-γ-aminobutyric acid (β-CPG, Baclofen, Lioresal), a derivative of γ-aminobutyric acid (GABA), is better able to penetrate the blood-brain barrier than the latter on systemic administration and has proven to be effective in alleviation of spasticity resulting from a variety of neurological disorders[2].

Because of its structural resemblance to GABA, it was initially postulated that β-CPG might function by activating GABA receptors on neurons in the spinal cord, where it had been shown to reduce both monosynaptic and polysynaptic reflexes. Further experiments have rendered this explanation untenable as the actions of β-CPG were not antagonized by bicuculline and occurred in the absence of any pronounced alterations in the electrical properties of the motoneuron membrane[3–6]. An alternative explanation, namely that β-CPG depressed reflexes by a presynaptic mechanism, was suggested to account for the experimental findings[6,7].

It has recently been claimed that β-CPG is a specific antagonist of substance P and that the depression of

[1] Acknowledgments. The author is indebted to Ciba-Geigy (Canada) Ltd. for a gift of β-CPG. BRENDA ROBSON provided technical assistance. This work was supported by grants from the Canadian M.R.C. and the University of Saskatchewan.

[2] W. BIRKMAYER, *Spasticity – a Topical Survey* (Huber, Bern 1972).
[3] F.-K. PIERAU and P. ZIMMERMANN, Brain Res. *54*, 376 (1973).
[4] J. DAVIES and J. C. WATKINS, Brain Res. *70*, 501 (1974).
[5] D. R. CURTIS, C. J. A. GAME, G. A. R. JOHNSTON and R. M. MCCULLOCH, Brain Res. 70, 493 (1974).
[6] R. A. DAVIDOFF and E. S. SEARS, Neurology *24*, 957 (1974).
[7] F.-K. PIERAU, G. K. MATHESON and R. D. WURSTER, Expl Neurol. *48*, 343 (1975).



A) Ratemeter recording of the firing of a single corticospinal neuron. Substance P (SP, 40 nA) and acetylcholine (A, 40 nA) excitation of this neuron, as well as its spontaneous discharge, were substantially depressed during the application of β-chlorophenyl-GABA (β-CPG, 2 nA). Recovery occurred rapidly once the β-CPG application was terminated. Periods of drug application are indicated by the horizontal bars above the trace. The ordinate is calibrated in spikes per second. B) Ratemeter recording of an unidentified cortical neuron. Acetylcholine (A, 40 nA) and L-glutamate (G, 40 nA) excited this cell. During the application of β-CPG Ach excitation, but not that evoked by L-glutamate, was antagonized. Recovery of the Ach response is apparent shortly after the termination of the β-CPG application.

monosynaptic reflexes by β-CPG therefore constitutes further evidence in support of the hypothesis that substance P is the transmitter released by primary afferent fibres[8]. Neurons in the rat cerebral cortex can be excited by substance P, acetylcholine (Ach) and L-glutamate[9,10]. In the present experiments the specificity of β-CPG antagonism of substance P has been evaluated on these neurons, utilizing Ach- and L-glutamate-induced excitations for comparisons.

Methods. 6 male Sprague-Dawley rats were anaesthetized with methoxyflurane, nitrous oxide and oxygen and mounted in a stereotaxic frame as described previously[10]. An electrode was placed in the ipsilateral medullary pyramidal tract for identification of corticospinal neurons. Extracellular action potentials of neurons in the sensorimotor cortex were recorded through the centre barrel (2 *M* NaCl) of seven-barrel micropipettes. The remaining barrels were filled by centrifugation with various combinations of the following drug solutions: acetylcholine chloride (0.1 *M*, pH 5.0), L-glutamate (0.2 *M*, pH 8.0), substance P (0.0008 *M*, pH 6.5, Beckman), GABA (0.2 *M*, pH 6.0), β-CPG (0.04 *M*, pH 3.5), NaCl (2 *M*). Antidromic stimulation of the pyramidal tract was used to identify corticospinal neurons by previously established criteria[10].

Results. The majority of these studies were conducted on deep, spontaneously firing cortical neurons, including identified corticospinal neurons. In confirmation of previous observations, Ach and substance P excited cells of this type. Of the 47 unidentified neurons tested with both substances, 31 (66%) were excited by substance P and 39 (83%) by Ach. Substance P excited 25 (93%) and Ach 26 (96%) of the 27 identified corticospinal neurons tested. The potency of substance P as an excitant showed some variability from electrode to electrode and its effects were frequently less pronounced than those of Ach applied from another barrel of the same electrode. Excitations evoked by both substances usually had a longer latency than that observed for L-glutamate-elicited excitation, and that initiated by substance P took one or more minutes to subside.

β-CPG administered by cationic currents (1–30 nA) reversibly reduced the firing rate of spontaneously active or drug-excited neurons. In comparison with GABA, the development of β-CPG's effect was slow (in excess of 1 min) and recovery was prolonged, frequently taking 1 to 3 min. If sufficient time was allowed for the development of a maximal effect, β-CPG appeared to be almost equipotent with GABA as a depressant on the basis of equivalent electrophoretic currents. β-CPG was tested on 28 unidentified neurons and 12 identified corticospinal cells. As is illustrated in Figure A, no selectivity could be demonstrated between the depressant effects of β-CPG on spontaneous, Ach and substance P evoked excitation. In every instance a reduction in substance P-evoked firing was paralleled by that in the Ach-induced response. The recovery curves were also comparable. Excitation evoked by pulses of L-glutamate was less affected by β-CPG (see Figure B), larger application currents being necessary to block the glutamate effect.

Discussion. The present observations confirm previous findings of a depressant action of electrophoretically applied β-CPG on cerebral cortical neurons[4,5]. No evidence was obtained that β-CPG is able to selectively antagonize substance P-induced excitation of cerebral cortical neurons, as Ach excitation was always affected to a comparable extent. Excitation evoked by L-glutamate was relatively resistant to depression by β-CPG, an observation that is consistent with findings on isolated perfused amphibian and mammalian spinal cords[6,8].

The precise mechanism of action of β-CPG remains to be elucidated. Since the GABA antagonist, bicuculline, does not reduce the effect of β-CPG[4,5], activation of GABA receptors seems to be an unlikely explanation for its depressant action. β-CPG depresses excitatory postsynaptic potentials in the absence of any changes in membrane excitability[3], an observation which, taken in conjunction with the present findings, suggests that at least part of its action may be to interfere with the effects of various putative excitatory transmitter agents.

[8] K. SAITO, S. KONISHI and M. OTSUKA, Brain Res. *97*, 177 (1975).
[9] J. W. PHILLIS and J. J. LIMACHER, Brain Res. *69*, 158 (1974).
[10] J. W. PHILLIS and J. J. LIMACHER, Expl Neurol. *43*, 414 (1974).

A new Wave (2nd c-Wave) on Corneoretinal Potential

T. NIKARA, S. SATO, T. TAKAMATSU, R. SATO and T. MITA

Department of Physiology, School of Medicine, Iwate Medical University, Morioka 020 (Japan), 25 November 1975.

Summary. The 2nd c-wave is a new wave of corneoretinal potential which is an on-response with a long latency (65–98 sec), and appears following the end of the c-wave of ERG. It is suggested that the 2nd c-wave is based on the tail of the late receptor potential of the retina.

We describe here a new wave of the corneoretinal potential which is an on-response and appears within a certain period, ranging from the end of the c-wave of the electroretinogram (ERG) to the light peak of the corneoretinal standing potential. The wave was tentatively termed by us '2nd c-wave', for the following reason: – the amplitude of the 2nd c-wave increases frequently with increase in that of the c-wave and vice versa[1].

Twenty adult cats were used. Before recordings, the cat was kept in darkness for 30 min. The corneoretinal potential was picked up by 2 Zn-$ZnSO_4$ nonpolarizing electrodes with cotton wicks between the cornea and the incized skin of the upper lid. The potential was then led to a pen-oscillograph through a high impedance DC amplifier. Anesthesia was maintained with nitrous oxide in oxygen (80%/20%) after initial preparation with halothane (2%). Paralysis of the eye muscles and the maintenance of ventilation and temperature have been described elsewhere[2]. The pupil was dilated with 1% atropine sulfate. The cornea was protected by a contact lens from drying out. The light from a xenon arc lamp was directed to the eye by means of Maxwellian view. The intensity of the light was 1000 lx at the corneal surface and varied by use of neutral density filtres. The light duration was provided by an electromagnetic shutter.

Four traces of the corneoretinal potential shown in Figure 1 differ from each other as to the c-wave. The 1st trace (1) illustrates the most common shape. The 3rd and