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GABA-A and GABA-B Receptors in the Cuneate Nucleus of the Rat *in vivo* *

P. Orviz, B. G. Cecchini and F. Andrés-Trelles **

Departamento de Farmacología y Terapéutica
Facultad de Medicina
33006 Oviedo (Spain)

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Electric stimulation of the rat forepaw evokes a negative potential (N-wave) at the ipsilateral cuneate nucleus. The responses of the N-wave to microiontophoretically applied GABA agonists and antagonists have been studied. Applications of GABA-A agonists (3-aminopropanesulfonic acid and muscimol) reduce the amplitude of the N-wave. This effect decreases during prolonged application, suggesting a desensitization of GABA-A receptors. In addition the effect of muscimol is reduced by (–)-bicuculline methiodide. Baclofen (a GABA-B agonist) also depresses the N-wave but its action lasts longer, is less reversible, shows no desensitization and is not blocked by (–)-bicuculline methiodide. The different responses of the N-wave to GABA-A and GABA-B agonists are compatible with the existence of different types of functional receptors for them in the cuneate nucleus of the rat. The receptors activated by muscimol (GABA-A) are clearly not the same as the ones activated by baclofen (conceivably GABA-B).

Key words: GABA receptors *in vivo*, GABA-A, GABA-B, Muscimol, Baclofen, Cuneate nucleus.

Receptors for the inhibitory neurotransmitter γ-aminobutyric acid (GABA) are not homogeneous. Two distinct types have been described in the mammalian Central Nervous System (CNS), GABA-A and GABA-B (3, 4, 16, 18, 19). GABA-B receptors are insensitive to GABA-A antagonists, such as bicuculline and picrotoxin, and baclofen (β-chlorophenyl-GABA, a liposoluble GABA-analogue) is the only known specific and potent GABA-B agonist (5, 8-10, 12, 15, 17).

Many studies on GABA receptors have been made *in vitro* using CNS slices or radioligands. The experiments described below study *in vivo* the effects of microiontophoretic applications of GABA agonists and antagonists on the negative potential (N-wave) evoked at cuneate nucleus of the rat by stimulation of afferents in the ipsilateral forepaw, in order to verify the presence of functional

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** To whom correspondence should be addressed.

GABA-A and GABA-A receptors in the nucleus.

Materials and Methods

Experiments were made in 70 male Wistar rats weighing between 250 and 350 g. Anaesthesia was induced with halothane (1-1.5 % in 30 % O_2 and 70 % N_2O) and maintained with intraperitoneal urethane (1.8 g/kg body weight). The head was fixed in a stereotaxic frame and the dorsal surface of the *medulla oblongata* exposed. The forepaw was stimulated at a rate of 1 Hz with supramaximal electric shocks (5 V) 0.2 ms wide through two stainless steel electrodes placed on the centre pad of the paw and under the skin of the forelimb.

The active recording electrode was one of the compartments of a multibarrelled glass micropipette (two to seven borosilicate barrels), filled with 3 M NaCl and containing a silver-silver chloride wire. Its D.C. impedance was lower than 10 MΩ. The tip of the micropipette (overall diameter 2-8 μm) was lowered progressively into the ipsilateral cuneate nucleus until it reached a depth of 400-800 μm. It is at this depth where microiontophoretic GABA typically produces greatest effects (2). The indifferent electrode was placed under the skin of the head. The bioelectric signal was led by a high impedance probe to a Grass P16 differential preamplifier, which filtered out frequencies higher than 1 KHz in order to exclude single action potentials. The D.C. output from the preamplifier was then led to a Neurolog System Digitimer amplifier and displayed on a Tektronix oscilloscope. The displays were photographed, either directly from the oscilloscope or after F.M. recording by a Hewlett-Packard 3964A recorder.

The remaining barrels of the micropipette were filled with acid solutions of the drugs, which were released from the tip of the micropipette by positive electric currents (lower than 200 nA) passing through the solution and generated by a five channel Analog-Medical System Corp. microiontophoresis pump. The following solutions were applied: GABA (Sigma) 200 mM in distilled water, pH 3.5; muscimol (Sigma) 50 mM in 165 mM NaCl, pH 3; 3-APS (3-aminopropanesulfonic acid, Sigma) 50 mM in 165 mM NaCl, pH 3; baclofen (Ciba-Geigy) 100 mM in 165 mM NaCl, pH 3; and (–)-bicuculline methiodide (Sigma) 10 mM in 165 mM NaCl, pH not adjusted.

Results

As microiontophoresis is only a semiquantitative technique, not being possible to measure the exact amount of drug released during an experiment, it is difficult to apply statistics to compare results from different experiments. Therefore, only those in which most of the variability sources were within certain specified limits were taken into account: adequate size and shape of control N-waves (amplitude higher than 300 μV and duration at half amplitude between 2.7 and 4.3 ms); absence of positive potentials (P-waves) after the N-wave; existence of conditioned inhibition (fig. 1B); and regular response to microiontophoretic GABA (2).

Microiontophoretic applications of the GABA-A agonist muscimol reduced the amplitude of the N-wave. When the ejection current was high enough (usually higher than 40 nA) an inversion of the N-wave, which became positive, appeared. After having reached a maximum in about 30-35 s the effect partially reverted, in spite of continuing the application of muscimol (fig. 1A). Once the ejection current was interrupted the N-wave recovered in a few seconds its control amplitude. This effect was mimicked by 3-APS, another potent GABA-A agonist. Previous and simulta-

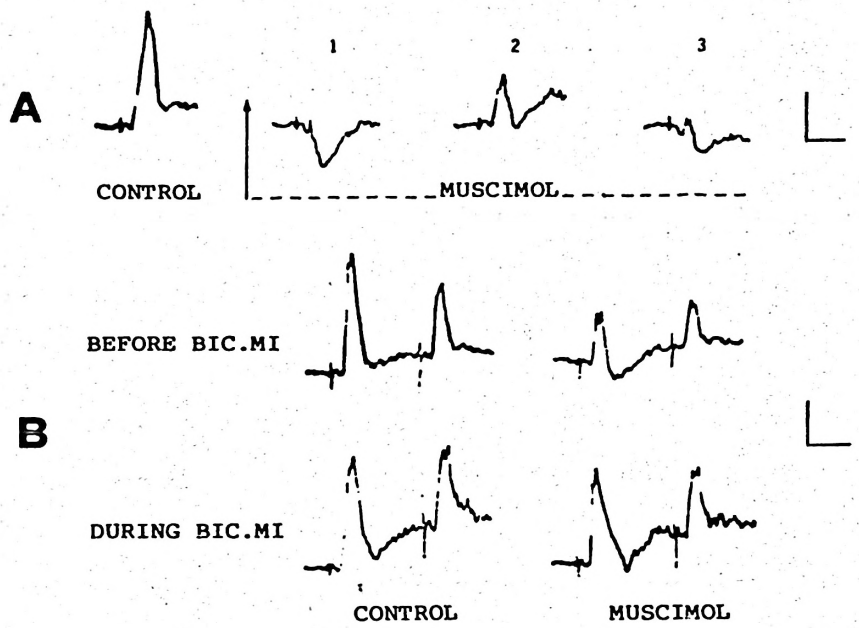

Fig. 1. *Effect of muscimol on the N-wave evoked at the cuneate nucleus of the rat.* A: Continuous microiontophoretic application of muscimol (60 nA). Records were made 30 s (1), 1 min (2) and 5 min (3) after starting muscimol release. Vertical calibration bar = 375 μV negative upwards and horizontal bar = 8 ms. B: Comparison between the responses of the N-wave to muscimol (20 nA for 3 min) in the absence and in the presence of (–)-bicuculline methiodide (BIC.MI, 60 nA for 6 min). This figure shows the negative waves evoked by two consecutive identical stimuli. The second N-wave is smaller, due to a gabergic *conditioned* inhibition. In this way it is checked that (–)-bicuculline methiodide is effective, since this GABA antagonist reduces the *conditioned* inhibition. Vertical calibration bar = 375 μV negative upwards and horizontal bar = 8 ms.

neous microiontophoretic (–)-bicuculline methiodide diminished the response of the N-wave to muscimol and this action depended on the ejection current (fig. 1B).

Microiontophoretically applied baclofen also depressed and, with higher current, inverted the N-wave (fig. 2A). The maximal effect appeared in less than 1 min and lasted the whole application of the drug. Recovery postbaclofen was not instantaneous and took nearly 3 min. When (–)-bicuculline methiodide was previous and simultaneously applied the effect of baclofen was unaffected (figure 2B).

Discussion

Gabergic mechanisms as well as presynaptic and postsynaptic inhibitions mediated by GABA have been repeatedly reported in the mammalian cuneate nucleus, both *in vivo* and *in vitro* (1, 2, 6, 7, 11, 13, 20). Our experimental model has enabled us to study the responses of the N-wave to locally applied GABA-A and GABA-B agonists. The differences described here are best explained assuming the existence of more than one kind of functional GABA receptor *in vivo*.

The involvement of GABA-A receptors is clear. The effects of muscimol and

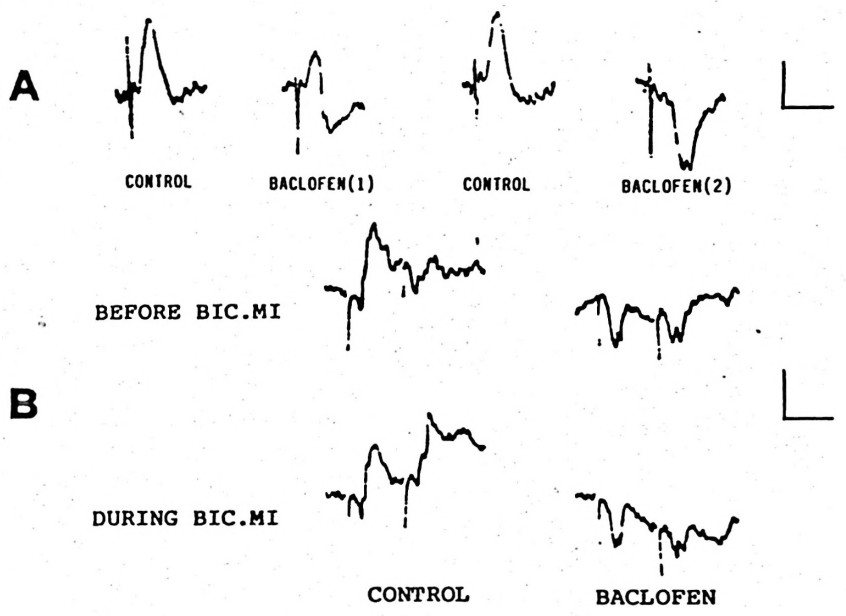

Fig. 2. *Response of the N-wave to baclofen.*
A: Relation between effect and ejection current. The response to 100 nA (1) is smaller than to 200 nA (2). Vertical calibration bar = 300 μV negative upwards and horizontal bar = 12 ms. B: For the same reasons as in fig. 1, the forepaw of the rat was stimulated with two consecutive identical stimuli. The effect of baclofen (40 nA for 1 min) in the absence of (–)-bicuculline methoiodide (BIC.MI) is compared with the response of the N-wave to baclofen (again 40 nA for 1 min) during simultaneous application of BIC.MI (30 nA for 6 min). Vertical calibration bar = 175 μV negative upwards and horizontal bar = 12 ms.

3-APS are rapidly reversible and similar to the ones produced by GABA (2). During maintained microiontophoretic application their inhibitory action is most pronounced initially and diminishes with time. Our observations agree with those made in other preparations by KRNJEVIC, who noted that the effect of GABA tends to *fade* (14). The fact that muscimol and 3-APS also show this characteristic phenomenon in the cuneate nucleus indicates that it might be due to a desensitization of GABA receptors, rather than to a common uptake mechanism for GABA-A agonists or to artifactual circumstances. Moreover, (–)-bicuculline methiodide blocks the effect of muscimol in a current-dependent way, confirming the action of this agonist on GABA-A receptors.

On the other hand, the characterization of GABA-B receptors is more difficult and incomplete, as specific antagonists are not available. Nevertheless, baclofen provokes a different sort of depression of the N-wave, less reversible than the response to GABA-A agonists. In addition, neither desensitization *(fading)* appears, nor (–)-bicuculline methiodide modifies its lasting effect, even with high ejection currents. It is thus highly likely that bacloren activaates receptors other than GABA-A, conceivably GABA-A.

Resumen

La estimulación eléctrica de la pata anterior de la rata provoca un potencial evocado negativo (onda

N) en el núcleo cuneiforme homolateral. Se han estudiado las respuestas de la onda N a la administración microiontoforética de agonistas y antagonistas del GABA. La administración de agonistas GABA-A (ácido 3-aminopropanosulfónico y muscimol) disminuye la amplitud de la onda N y este efecto decrece a lo largo de una administración prolongada, lo que sugiere una desensibilización de los receptores GABA-A. Además, el (–)-metilyoduro de bicuculina disminuye el efecto del muscimol. El baclofén (agonista GABA-B) también reduce la onda N pero su acción es más duradera y menos reversible, no sufre una desensibilización y no es antagonizada por el (–)-metilyoduro de bicuculina. Las distintas respuestas de la onda N a los agonistas GABA-A y GABA-B son compatibles con la existencia para ellos de distintos tipos de receptores funcionales en el núcleo cuneiforme de la rata. Los receptores activados por el muscimol (GABA-A) son claramente diferentes de los activados por el baclofén (presumiblemente GABA-B).

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