Br. J. Pharmac. (1978), **63**, 503–507

EFFECTS OF γ-AMINOBUTYRIC ACID ON NERVE TERMINAL EXCITABILITY IN A SLICE PREPARATION OF CUNEATE NUCLEUS

ANN G. HAYES & M.A. SIMMONDS

Department of Pharmacology, The School of Pharmacy,
University of London, 29/39 Brunswick Square, London WC1N 1AX

1 Superfusion of a slice preparation of the rat cuneate nucleus with γ-aminobutyric acid (GABA) depolarized the afferent nerve fibres and increased their excitability. However, before the depolarization had reached its peak the increased excitability reversed to a decreased excitability, an effect which outlasted the depolarization.

2 Both components of the GABA excitability response were dose-related, Cl^--dependent and antagonized by bicuculline.

3 Possible mechanisms underlying the sequence of excitability changes are discussed.

Introduction

It has been shown in the preceding paper (Simmonds, 1978) that a slice containing the cuneate nucleus of the rat can be used to study the presynaptic actions of γ-aminobutyric acid (GABA). Thus, GABA produced a dose-related depolarization of the cuneate afferent fibres. This depolarization was chloride-dependent and was antagonized by various convulsants, including bicuculline.

In cat cuneate nucleus, *in vivo*, the depolarization of nerve terminals during presynaptic inhibition was always accompanied by an increase in the excitability of the terminals (Andersen, Eccles, Schmidt & Yokota, 1964). Subsequently, it has been shown that this increase in excitability is antagonized by bicuculline and picrotoxin (Banna & Jabbur, 1969; Banna, Naccache & Jabbur, 1972; Levy & Anderson, 1972) and is, therefore, thought to be GABA-mediated.

The purpose of the present experiments was to determine whether the GABA-mediated depolarization of afferent nerves in the cuneate nucleus slice was accompanied by a change in their excitability. In this *in vitro* preparation, the actions of drugs and changes in the ionic environment could be more readily studied than was possible *in vivo* (Hayes, Gartside & Straughan, 1977).

Methods

A slice containing the cuneate nucleus and dorsal funiculus was prepared as described in the previous paper (Simmonds, 1978). The perfusion arrangement was slightly modified from that described previously by the inclusion of an extra perfused compartment between the suction electrode and the main body of the slice (Figure 1). The terminal region of the afferent pathway was stimulated through a glass microelectrode filled with 4 M NaCl (tip diameter 15 μm, resistance 0.3 MΩ). Stimuli were single rectangular pulses, width 100 to 200 μs and frequency 0.2 hertz. The voltage was adjusted to evoke approximately half-maximal antidromic action potentials; the current required was in the range 10 to 60 μA, but presumably much of this leaked through the bathing medium. The action potentials were recorded via two Ag/AgCl electrodes mounted in agar-saline and contacting respectively compartment (2) of the bath and the suction electrode. Four consecutive potentials were led through a differential amplifier (time constant 1 s), averaged on a Neurolog Averager and the average potential copied on a Medelec u.v. recorder. Alternatively, single potentials were fed into a Peak Height Detector (Courtice, 1977), which detects the peak of a potential and then holds the signal for sufficient time to write it out on a chart recorder (e.g. see Figure 2b). The action potentials were also monitored on an oscilloscope to establish that the latency to peak remained constant throughout an experiment. The d.c. signal between compartments (1) and (2) was recorded differentially via Ag/AgCl electrodes and written out on a chart recorder. Responses to GABA and K^+ were measured at their peak in the control period. Thereafter, the responses were measured at the same intervals from the onset of agonist application.

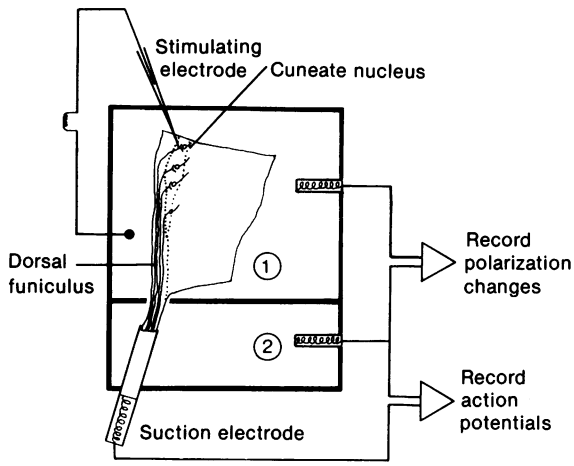

Figure 1 Diagram of the cuneate nucleus slice showing the arrangements for stimulation and recording. Both compartments (1) and (2) were perfused, but drugs were added to compartment (1) perfusate only.

Results

Resting excitability

Stimulation within the terminal region of the cuneate nucleus produced an antidromic compound action potential which was recorded near the cut end of the tract as a monophasic, positive-going spike (Figure 2a). It was sometimes followed by a small negative potential. In individual experiments, the latency to the peak of the potential remained constant, being in the range 0.7 to 1.4 ms; also the duration remained constant, in the range 1 to 1.5 milliseconds. The size of the potential was within the range 500 μV to 3 millivolts.

Effects of GABA

Perfusion of the main body of the slice with GABA caused a shift in the d.c. signal indicating a depolarization of the afferent fibres (Figure 2b). This depolarization was sometimes followed by a small hyperpolarization. GABA also increased the amplitude of the submaximal antidromic action potential by 10 to 40%. However, while the fibres were still depolarized, the action potential amplitude then often decreased by 10 to 50% compared with the control (Figure 2b). This phase of decreased excitability outlasted the depolarization and often extended up to 30 minutes. During this phase, the latencies to onset and peak of the potential remained the same as during the control period. The area of the potential was decreased, which suggests that the decreased amplitude was not principally due to temporal dispersion. The decrease in excitability was also not correlated with the occasional appearance of a hyperpolarization after GABA. The threshold concentration of GABA for producing a measurable change in excitability was about 5×10^{-5} M, which is somewhat higher than the threshold concentration of 10^{-5} M for producing a depolarization (Simmonds, 1978). All three GABA effects were dose-related.

Effect of increasing the K^+ concentration

Increasing the K^+ concentration of the Krebs perfusing the main body of the slice from 3 mM to 9 mM depolarized the afferent fibres and increased the amplitude of the stimulus-evoked antidromic potentials. The time courses for the two effects were approximately the same and there was no subsequent decrease in amplitude of the antidromic potential.

Effect of glutamate

The effect of perfusing the main body of the slice with glutamate was also examined. Glutamate had no effect at 10^{-4} M and 10^{-3} M. At 10^{-2} M, it produced a very small depolarization, but no detectable effect on excitability.

Effect of low chloride

It has been reported that the depolarizing effects of GABA are due to an increased conductance to Cl^- (Nishi, Minota & Karczmar, 1974; Adams & Brown, 1975; Deschenes, Feltz & Lamour, 1976). This was manifest as an enhanced GABA response after brief exposure to low Cl^-, but as a decreased GABA response after prolonged exposure to low Cl^-. Thus, the effect of replacing 80% of the Cl^- in the Krebs solution with isethionate was studied in five experiments.

At 15 to 30 min after the changeover, depolarizations and changes in excitability due to 10^{-3} M GABA were both decreased in amplitude (Table 1). Upon restoration of normal Krebs solution, partial recovery was obtained. GABA was not tested immediately after Cl^- replacement to allow the resting excitability to stabilize after a transient increase of 30–80%.

Both the depolarization and increase in excitability produced by 9 mM K^+ were enhanced in low Cl^- Krebs solution (Table 1).

Effects of (+)-bicuculline

Figure 3 shows the effect of increasing concentrations of (+)-bicuculline from 10^{-6} M to 10^{-5} M on the

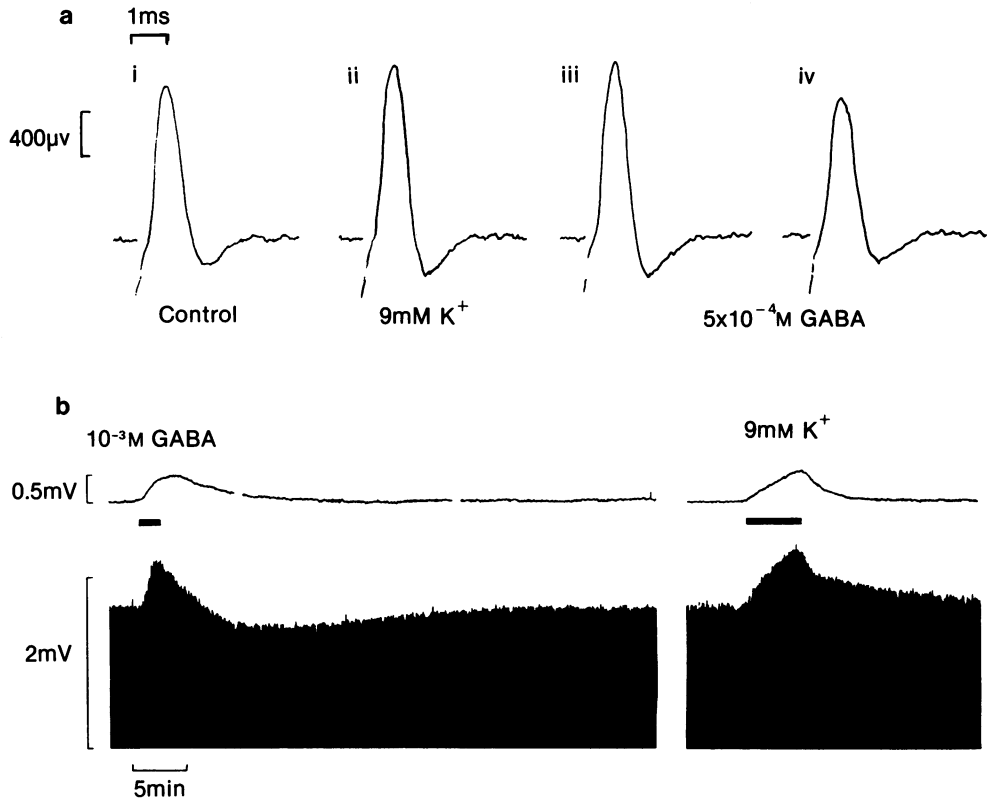

Figure 2 (a) Antidromic response produced by stimulation in the region of the afferent terminals in the cuneate nucleus slice, recorded near the cut end of the tract as a mass action potential: (i) control; (ii) potential of increased amplitude 4 min after the onset of a 5 min application of 9 mM K^+; (iii) potential of increased amplitude and (iv) potential of decreased amplitude at 1.5 min and 12 min, respectively, after the onset of a 2 min application of 5×10^{-4} M γ-aminobutyric acid (GABA). (b) A different experiment showing the effect of 10^{-3} M GABA and 9 mM K^+ on membrane polarization (above) and excitability (below) in the cuneate nucleus slice. The peak amplitude of the antidromic action potentials is represented in the lower trace by the upward deflections. The bars represent the contact time of the drugs.

Table 1 Effect of replacing 80% of the external chloride with isethionate on changes in excitability and membrane polarization produced by γ-aminobutyric acid (GABA) 10^{-3} M and K^+ 9 mM

		Control	*Low Cl^-*	*Recovery*	*n*
GABA	Excitability increase	100	41.3 ± 7.2	63.3 ± 7.6	5
	Excitability decrease	100	8.0 ± 8.0	85.1 ± 23.6	5
	Depolarization	100	60.5 ± 11.9	117.6 ± 26.3	5
K^+	Excitability increase	100	200, 166	29, 35	2
	Depolarization	100	124, 222	86, 83	2

The values are expressed as a % of the control responses. For the GABA experiments, mean and standard error values are shown. For the K^+ experiments, the two individual values are shown.

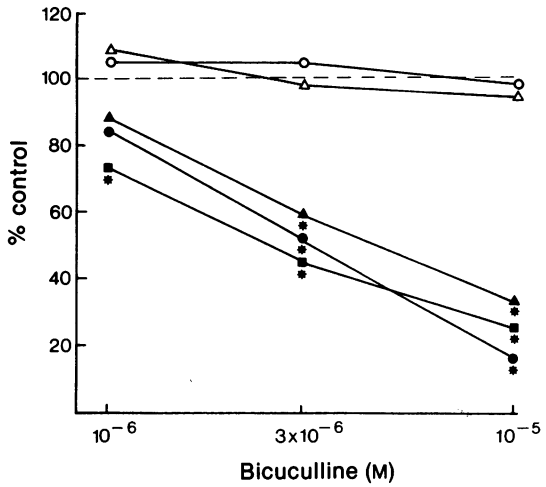

Figure 3 Effect of increasing concentrations of (+)-bicuculline on the responses to γ-aminobutyric acid (GABA) and K^+. Each point represents the mean value calculated from at least five experiments. Filled symbols represent responses to 5×10^{-4} M GABA and open symbols represent responses to 9 mM K^+; (▲) and (△) represent depolarization; (●) and (○) represent the increase in excitability; (■) represents the decrease in excitability. * indicates points significantly different from control i.e. 100% ($P < 0.05$), calculated by the non-parametric sign test.

responses to 5×10^{-4} M GABA and 9 mM K^+. (+)-Bicuculline itself had no consistent effects on polarization or excitability, and no effect on the K^+ responses. However, it produced a concentration-dependent reduction of all three components of the GABA response.

Discussion

Depolarization by GABA of the afferent fibres in the cuneate nucleus slice was associated with an increase in the excitability of the terminal regions of those fibres. The extent to which this action of GABA was confined to the nerve terminals or involved the pre-terminal axons as well, cannot be determined from these experiments. Nevertheless, the increase in excitability agrees with results reported in a number of different preparations (Schmidt, 1963; Davidson & Southwick, 1971; Barker & Nicoll, 1972; Curtis, Lodge & Brand, 1977). The GABA depolarization was not affected by high magnesium Krebs (Simmonds, 1978 and unpublished observations) and, therefore, was probably a direct effect. However, it was impossible to assess the effect of raising the magnesium concentration on the GABA-induced changes in excitability as the magnesium itself produced a large decrease in excitability.

L-Glutamate had no effect on excitability and only produced a very small depolarization at doses above 10^{-3} M. Davidson & Southwick (1971) reported an increase in cuneate afferent terminal excitability *in vivo* with topical glutamate. Also, Curtis *et al.* (1977) reported an increased excitability of feline dorsal root afferents *in vivo* with iontophoretic glutamate. These data suggest two possibilities; either that there was a selective loss of glutamate responses in the slice preparation, or, more likely, that there are few glutamate receptors on cuneate afferents, similar to the situation for the cell bodies of these neurones (De Groat, Lalley & Saum, 1972).

GABA also produced a second effect on excitability —the prolonged phase of decreased excitability. This phase could not be secondary to the depolarization, as high K^+ often produced an equivalent or larger depolarization than GABA, but only an increase in excitability. This effect of GABA in decreasing excitability has not been previously described in nerve terminals. However, in cell bodies GABA-induced depolarizations were accompanied by a decreased excitability measured intracellularly, e.g. in rat dorsal root ganglion cell bodies, which give rise to the cuneate afferents (Lawson, Biscoe & Headley, 1976; Deschenes *et al.*, 1976) and also in rat superior cervical ganglion cell bodies (Adams & Brown, 1975). The latter authors explain the decreased excitability as a shunting effect due to increased Cl^- conductance. As the excitability decrease in the present experiments was Cl^--dependent, a similar explanation could be offered. The fact that the excitability decrease outlasted the depolarization suggests that the latter may have been limited by loss of internal Cl^-.

We would like to thank Professor D.W. Straughan for his criticisms of the manuscript. This work was partly supported by an M.R.C. project grant. A.G.H. is an S.R.C. Scholar.

References

ADAMS, P.R. & BROWN, D.A. (1975). Actions of GABA on sympathetic ganglion cells. *J. Physiol.*, **250**, 85–120.

ANDERSEN, P., ECCLES, J.C., SCHMIDT, R.F. & YOKOTA, T. (1964). Depolarization of presynaptic fibres in the cuneate nucleus. *J. Neurophysiol.*, **27**, 92–106.

BANNA, N.R. & JABBUR, S.J. (1969). Pharmacological studies on inhibition in the cuneate nucleus of the cat. *Int. J. Neuropharmac.*, **8**, 299–307.

BANNA, N.R., NACCACHE, A. & JABBUR, S.J. (1972). Picrotoxin-like action of bicuculline. *Eur. J. Pharmac.*, **17**, 301–302.

BARKER, J.L. & NICOLL, R.A. (1972). GABA: role in primary afferent depolarization. *Science, N.Y.*, **176**, 1043–1045.

COURTICE, C.J. (1977). A circuit for recording evoked action potential amplitudes. *J. Physiol.*, **268**, 1–2P.

CURTIS, D.R., LODGE, D. & BRAND, S.J. (1977). GABA and spinal afferent terminal excitability in the cat. *Brain Res.*, **130**, 360–363.

DAVIDSON, N. & SOUTHWICK, C.A.P. (1971). Amino acids and presynaptic inhibition in the rat cuneate nucleus. *J. Physiol.*, **219**, 689–708.

DE GROAT, W.C., LALLEY, P.M. & SAUM, W.R. (1972). Depolarization of dorsal root ganglia in the cat by GABA and related amino acids: antagonism by picrotoxin and bicuculline. *Brain Res.*, **44**, 273–277.

DESCHENES, M., FELTZ, P. & LAMOUR, Y. (1976). A model for an estimate *in vivo* of the ionic basis of presynaptic inhibition: an intracellular analysis of the GABA-induced depolarization in rat dorsal root ganglion. *Brain Res.*, **118**, 486–493.

HAYES, A.G., GARTSIDE, I.B. & STRAUGHAN, D.W. (1977). Effect of four convulsants on the time course of presynaptic inhibition and its relation to seizure activity. *Neuropharmac.*, **16**, 725–730.

LAWSON, S.N.,, BISCOE, T.J. & HEADLEY, P.M. (1976). The effect of electrophoretically applied GABA on cultured dissociated spinal cord and sensory ganglion neurones of the rat. *Brain Res.*, **117**, 493–497.

LEVY, R.A. & ANDERSON, E.G. (1972). The effect of the GABA antagonists bicuculline and picrotoxin on primary afferent terminal excitability. *Brain Res.*, **43**, 171–180.

NISHI, S., MINOTA, S. & KARCZMAR, A.G. (1974). Primary afferent neurones: the ionic mechanism of GABA-mediated depolarization. *Neuropharmac.*, **13**, 215–219.

SCHMIDT, R.F. (1963). Pharmacological studies on the PAD of the toad spinal cord. *Pflugers Arch.. ges. Physiol.*, **277**, 325–346.

SIMMONDS, M.A. (1978). Presynaptic actions of γ-aminobutyric acid and some antagonists in a slice preparation of cuneate nucleus. *Br. J. Pharmac.*, **63**, 495–502.

(Received November 14, 1977.
Revised February 8, 1978.)