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An unusual effect of γ-aminobutyric acid on synaptic transmission of frog tectal neurones *in vitro*

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1 Bath-applied γ-aminobutyric acid (GABA) enhanced, in a dose-dependent fashion, the amplitude of optic nerve-evoked monosynaptic excitatory responses of the frog optic tectum superfused *in vitro* at 7°C.

2 Muscimol was more potent than GABA in eliciting similar effects.

3 GABA-induced responses were antagonized by picrotoxin and were insensitive to bicuculline or strychnine.

4 Raising the bath temperature to 20°C reduced the potency of GABA on these preparations.

5 No significant effect of GABA on the compound action potential of the whole optic nerve was found.

6 These data indicate that GABA can amplify visual inputs to the tectum through bicuculline-insensitive mechanisms.

Introduction

In the vertebrate brain γ-aminobutyric acid (GABA) is believed to be the major inhibitory neurotransmitter acting via bicuculline-sensitive receptors (Iversen, 1984). There are, however, exceptions to this rule: for instance, when GABA is applied to dendrites of hippocampal neurones, a bicuculline-sensitive facilitation of excitatory synaptic potentials may be observed (Andersen *et al.*, 1980). Here we describe a novel effect of GABA, namely a bicuculline-insensitive potentiation of excitatory transmission on optic tectum neurones of the frog brain.

Methods

Following several days of exposure to a 12 h light/dark cycle at 7°C, light-adapted frogs (*Rana temporaria*) were anaesthetized with tricaine (0.1%), their whole brain was removed and placed in a 2 ml bath at 7°C. The preparation was superfused at a rate of about 10 ml min^{-1} with precooled Ringer solution of the following composition (mM): NaCl 111, KCl 2.5, $NaHCO_3$ 10, $NaH_2PO_4.2H_2O$ 0.1, $CaCl_2$ 2 and glucose 4 (gassed with 95% O_2/5% CO_2). A microelectrode containing 3 M NaCl was placed in the optic tectum to record field potentials evoked by electrical stimulation (0.08 Hz; 1 ms; varying intensity) of the contralateral optic nerve. Field potential responses were recorded via a d.c. amplifier (0–3 kHz), digitized by a Datalab transient recorder and played back on to a pen recorder. Compound action potentials of the optic nerve were recorded with a microelectrode inserted into the nerve just before its chiasmatic decussation. All drugs were dissolved in Ringer solution and applied via precooled flowlines. In order to check for the depolarizing activity of high K^+ solutions, the concentration of this ion in the bathing medium was raised to 14.5 mM. Dilutions of bicuculline were prepared immediately before use from fresh stock solutions (2 mM) of the free base dissolved in 0.02 N HCl. Results are expressed as mean ± s.e.mean. Statistical comparison of data was carried out with the Friedman non-parametric two way analysis of variance (see Colquhoun, 1971) with $P < 0.05$ taken as indicative of a significant difference.

Results

The data were obtained from 33 preparations surviving for at least 6 h. With the recording electrode placed at 50–100 μm depth from the pial surface, optic nerve stimulation (either with submaximal or with supramaximal intensity) evoked two characteristic

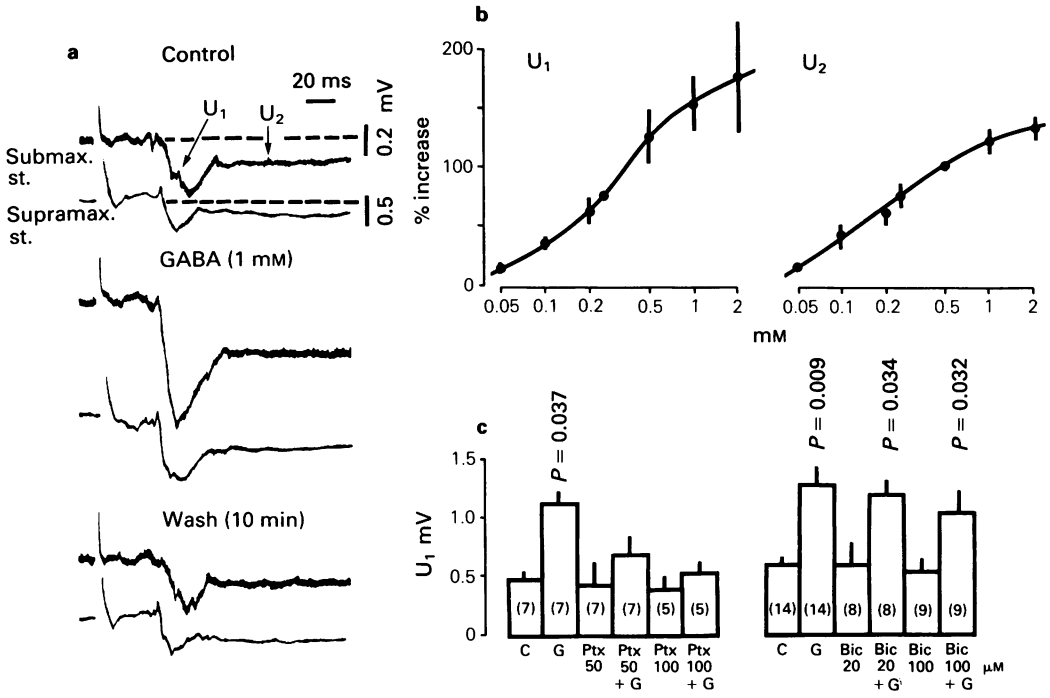

Figure 1 Effects of GABA on optic nerve-evoked synaptic responses of the frog optic tectum. (a) Pairs of d.c. oscilloscope records (note that in each pair top trace has higher amplification than bottom one) obtained following submaximal (Submax st.) or supramaximal (Supramax. st.) stimulation of the contralateral optic nerve. U_1 and U_2 postsynaptic waves are indicated by arrows. GABA (1 mM; 60 s superfusion; see middle row) enhanced these waves which recovered after wash (bottom row). (b) GABA log concentration-response curves for U_1 (left) and U_2 (right) waves. Data are from 4 experiments in which each concentration was tested at least twice in ascending and descending order. Responses are expressed as % increase in peak amplitude of waves evoked by supramaximal stimuli. When s.e.mean are < than symbols, they are not shown. Curves were fitted by eye and their apparent ED_{50} values were estimated as concentrations producing 50% of the apparent maximal response. (c) Histograms of the effects of GABA (G; 1 mM), picrotoxin (Ptx; 50 or 100 μM) and bicuculline (Bic; 20 or 100 μM) on U_1 wave amplitude (C is control wave). After obtaining responses to GABA in control Ringer solution, preparations were exposed to picrotoxin or bicuculline and GABA retested after at least 30 min. Number of experiments in parentheses. *P* values are shown for each data group significantly different from its preceding group. Note that picrotoxin or bicuculline *per se* produced no significant effects on field potentials.

monosynaptic negative waves (U_1 and U_2; note that the sharp peak of the U_1 wave is a population spike; Figure 1a). In 29 out of 31 preparations GABA (0.05–2 mM) induced a concentration-related and reversible increase in the peak amplitude of both U_1 and U_2 waves with no apparent change in the latency for the onset of the submaximal or supramaximal U_1 wave (Figure 1a; the U_2 latency was difficult to measure as the early part of this wave overlapped the U_1 wave). As shown in Figure 1a, GABA also produced an 11% reduction in the time to peak of the submaximal U_1 wave (measured from the beginning of the stimulus artefact to the apex of this wave). Hence, in the presence of GABA the temporal characteristics of the submaximal U_1 wave closely resembled those of its supramaximal waveform whose time to peak value remained unchanged (supramaximal U_1 values of controls and GABA-treated tecta were 59 ± 1.4 and 58 ± 1.5 ms respectively). The enhancing effect of GABA did not fade over about 2 min application, was equally present with submaximal or supramaximal field potentials and was approximately equipotent for the U_1 and U_2 waves. Figure 1b shows log concentration-response plots for GABA acting on the U_1 and U_2 waves evoked by supramaximal pulses. From these curves the estimated ED_{50} (concentration producing half the apparently maximal effect) for GABA was 0.2 mM. Similar effects on supramaximally-stimulated field potentials were produced by the GABA analogue muscimol (0.2–50 μM). Figure 2 shows the concentra-

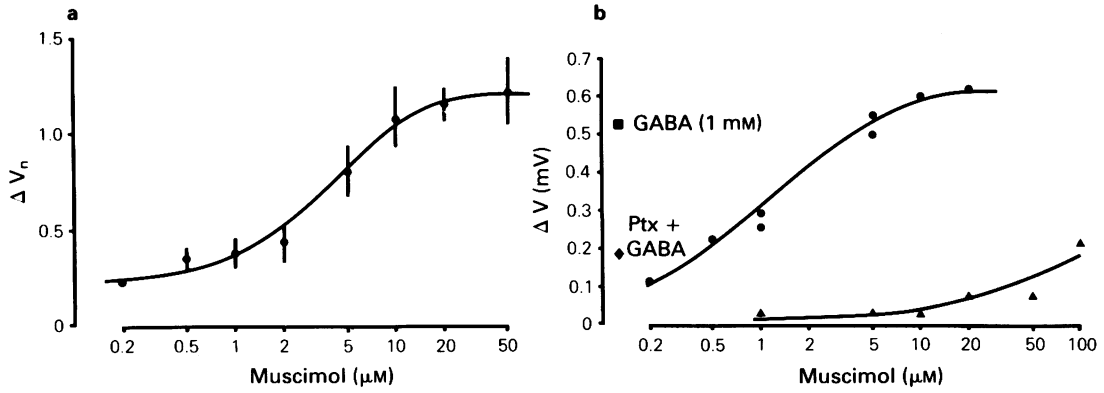

Figure 2 Effect of muscimol on optic nerve-evoked U_1 response. (a) Log muscimol concentration-response plot in which the increase in U_1 amplitude produced by each concentration of muscimol was normalized with respect to the increase evoked by 1 mM GABA on the same preparation. Data are from 4 experiments (further details in Figure 1 legend). (b) Plots of log muscimol concentration (abscissa scale) *vs.* the increase in U_1 amplitude (ordinate scale) in control Ringer solution (●) and in the presence of 100 μM picrotoxin (▲). Data are from one experiment in which responses to 1 mM GABA were also observed in control Ringer (■) or following exposure to picrotoxin (100 μM; ◆).

tion-response plot for the action of muscimol on the U_1 wave (estimated $ED_{50} = 2.7\,\mu M$). In order to compare the enhancing activity of muscimol vs. GABA, the data presented in Figure 2a were normalized with respect to the effect produced by 1 mM GABA in the same experiments. The maximum response induced by muscimol was similar to that produced by GABA. Muscimol also enhanced the U_2 wave amplitude in a concentration-dependent fashion; the resulting log concentration-response plot was sigmoidal and attained a maximum similar to that of the U_1 wave effect. The estimated ED_{50} value for muscimol action on the U_2 wave was 2.0 μM ($n = 4$). Field potential amplitudes were slightly enhanced at first and subsequently largely depressed (− 60% on average) by 14.5 mM K^+ ($n = 4$). L-Glutamate (2 mM) produced a transient increase in the field potentials (+26%) followed by a depression (−23%; $n = 8$). Only in two experiments did GABA reduce the U_1 and U_2 amplitudes (an effect replicated with muscimol on the same preparations). The enhancing effect of GABA on the U_1 and U_2 waves was blocked by picrotoxin

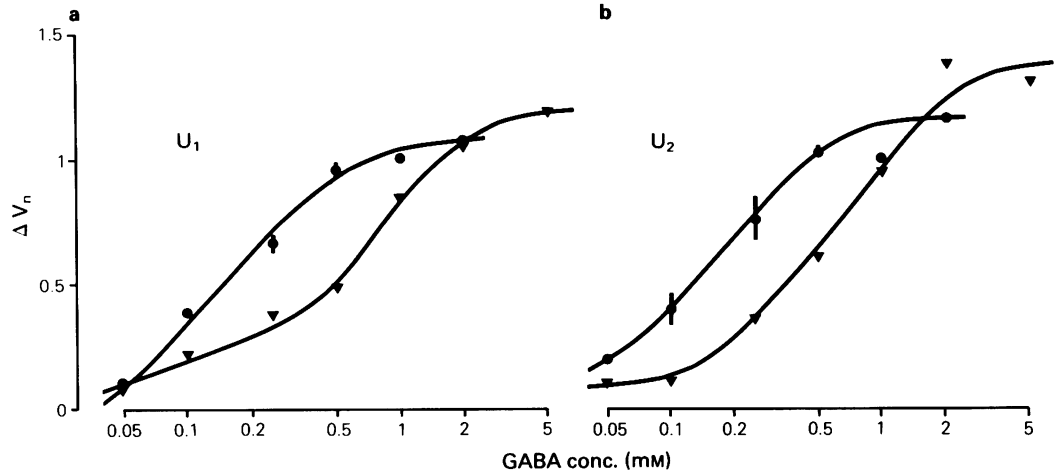

Figure 3 GABA concentration-response curves obtained at 7°C (●) and 20°C (▼). Responses are increases in supramaximal field amplitude for U_1 (a) and U_2 (b) waves and are expressed as data normalized with respect to the response induced by 1 mM GABA at 7°C. Applications of GABA were repeated up to three times and s.e.mean are shown when they are larger than symbols. All data are from the same preparation.

(50–100 μM) but not by bicuculline (20–100 μM) or strychnine (1 μM) (see Figure 1c for the U_1 wave data). Picrotoxin was also an effective antagonist of muscimol as illustrated in Figure 2b, where the muscimol concentration-response curve was strongly depressed and shifted to the right. In 2 experiments (one of them is depicted in Figure 3) concentration-response curves for GABA were constructed at 7°C as well as at 20°C: at the higher temperature the curves underwent a shift to the right with little change in the apparent maximum but a 2.5–3.5 fold increase in the estimated ED_{50} value. Finally, the effect of 1 mM GABA on the compound action potential of the optic nerve fibres was examined in a cold medium (7°C). In control conditions the compound action potential comprised four waveforms; the first one with a time to peak of less than 4 ms could not be easily separated from the stimulus artefact. The other three waveforms had amplitudes which obviously varied with the electrode location characteristics but had consistently similar time to peak values of 7 ± 1, 23 ± 3 and 46 ± 6 ms respectively ($n = 4$). GABA had no significant effect on the amplitudes of these waveforms or on the time to peak values (as they changed at most by only 2%). Exposure of optic nerve fibres to 14.5 mM K^+ produced a $39 \pm 10\%$ depression in the waveform amplitude of the compound action potentials preceded by an 11% reduction in the time to peak values.

Discussion

In the frog optic tectum the optic nerve fibres (possibly utilizing acetylcholine as neurotransmitter; see Freeman & Norden, 1984) establish monosynaptic excitatory connections with the dendrites of deep tectal neurones. Excitatory synaptic potentials (and population spike) have been recorded extracellularly *in vivo* as surface-negative field potentials (U_1 and U_2; cf. Chung *et al.*, 1974) elicited by either electrical optic nerve stimulation (Chung *et al.*, 1974) or specific visual signals presented to the animal's field of vision (Maturana *et al.*, 1960). In the present study based on recordings form the optic tectum of the amphibian brain *in vitro*, similar postsynaptic waveforms were reproducibly observed although they had a longer latency and time course and smaller amplitude (cf. Sivilotti, 1985) owing to the much lower temperature at which recordings were performed. The large enhancement by GABA of these responses was a novel phenomenon. This effect of GABA was concentration-dependent and yielded sigmoidal concentration-response curves at 7°C, a temperature which is known to depress the neuronal uptake system for this amino acid by the frog nervous tissue (Davidoff & Adair, 1975). At room temperature (20°C) the GABA curve was shifted to the right presumably because the operation of GABA transport processes reduced the amount of GABA present in the extracellular space. The field potential enhancement was apparently not due to an action of GABA on optic nerve fibres as GABA (1 mM) had only slight effects on the compound action potential of the optic nerve. It is however conceivable that GABA might have been acting on the optic nerve endings; this hypothesis can be tested by assessing changes in the excitability of these nerve terminals during GABA application. Nevertheless, it is unlikely that GABA was simply facilitating endogenous transmitter release since depolarizing concentrations of K^+ had little enhancing effects on field potentials before blocking them. Moreover, since the supramaximal field responses were also largely increased by GABA without significant alteration in their time to peak, it seems improbable that greater synchronization of trans-synaptically induced neuronal discharges was responsible for the action of GABA. Finally, there is no evidence that GABA itself might be the transmitter of the optic nerve (Freeman & Norden, 1984). We are therefore led to suggest either that GABA enhanced tectal neurone responses by suppressing inhibitory inputs, or that GABA directly excited tectal neurones. Intracellular recordings after block of synaptic transmission will be necessary to test these hypotheses. Regardless of the precise site of action of GABA, it is interesting to note that its effect was potently mimicked by the analogue muscimol, did not show fading, and was blocked by picrotoxin but not by bicuculline. Hence, GABA did not appear to act via $GABA_B$ receptors which are comparatively less sensitive to muscimol and not blocked by picrotoxin (Bowery *et al.*, 1983) or by conventional $GABA_A$ receptors which are rather sensitive to bicuculline (Nistri, 1983). In conclusion, the strong enhancement by GABA of monosynaptic transmission in the frog optic tectum reveals a novel unconventional way of amplifying synaptic inputs by using an amino acid which is generally considered to be an inhibitory neurotransmitter (Iversen, 1984).

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