Br. J. Pharmac. (1979), **65**, 223–228

PRESYNAPTIC γ-AMINOBUTYRIC ACID RESPONSES IN THE OLFACTORY CORTEX

HILARY G. PICKLES[1]

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

1 Potential changes were recorded from the lateral olfactory tract in slices of rat olfactory cortex *in vitro* at room temperature.

2 Superfused γ-aminobutyric acid (GABA) usually produced a dose-related depolarization of the lateral olfactory tract. Muscimol and 3-aminopropanesulphonic acid appeared more potent depolarizing agents than GABA, and glycine and taurine appeared less potent. Carbachol and glutamate were virtually ineffective.

3 The GABA responses were at least partially Cl^--dependent.

4 (+)-Bicuculline and higher concentrations of strychnine antagonized the GABA but not the glycine-induced depolarizations. Paradoxically, responses to high doses of GABA were sometimes potentiated by both bicuculline and strychnine.

5 It is suggested that GABA receptors could occur as widely on nerve terminals as they do postsynaptically in the CNS, where GABA could be involved in the modulation of transmitter output.

Introduction

γ-Aminobutyric acid (GABA) has been shown to depolarize dorsal roots (Levy, 1974) and dorsal funiculi (Simmonds, 1978), and this action may be related to its role in presynaptic inhibition. However, GABA also depolarizes at sites in the peripheral nervous system where it is unlikely to have a synaptic action (De Groat, 1970; Bowery & Brown, 1974; Brown & Marsh, 1978). Recently it was suggested that GABA receptors are also present on the terminal of the lateral olfactory tract (LOT) (Pickles & Simmonds, 1976). In the present study the lateral olfactory tract is also shown to be depolarized by GABA and this is the first clear demonstration of the presence of presynaptic GABA receptors in the cerebral cortex. It is suggested that presynaptic GABA receptors might be as widespread in the CNS as postsynaptic receptors and this could be important in the modulation of transmitter output.

A brief preliminary report of some of this work has been published (Simmonds & Pickles, 1978).

Methods

Slices of rat olfactory cortex, approximately 0.5 mm thick, were prepared and preincubated in modified Krebs medium at room temperature as previously described (Pickles & Simmonds 1976). A slice was then placed in a small chamber through which medium circulated, and the rostral end of the LOT, trimmed free of surrounding tissue, was passed through a small greased slot in a barrier into a second compartment of the bath, or else drawn up into a suction electrode. Recordings were made between two Ag/AgCl electrodes embedded in agar-saline, one of which was in contact with the compartment containing the slice and the cortical end of the tract, and the other was in contact with the second compartment or suction electrode containing the cut end of the LOT. The d.c. level between the two electrodes was amplified and written out on a chart recorder. Agonists were added to the medium bathing the main body of the slice for 3 min periods, with at least 15 min between doses. In experiments with antagonists, at least 30 min of equilibration took place in each antagonist concentration, before the next test agonist dose was given. Responses to drugs were measured at their peak amplitude from the projected baseline.

[1] Present address: Clinical Pharmacology Unit, The National Hospital, Queen Square, London WC1N 3BG.

0007-1188/79/020223-06 $01.00

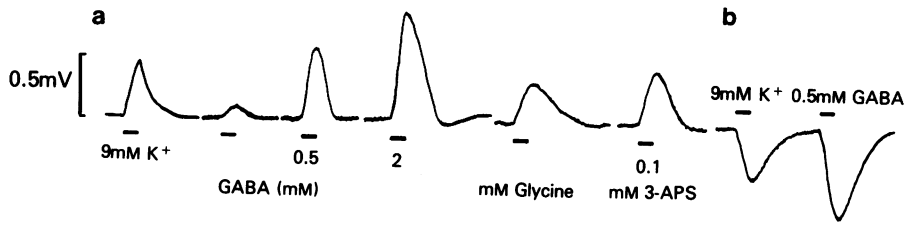

Figure 1 γ-Aminobutyric acid (GABA) evoked depolarizations in the lateral olfactory tract (LOT). Horizontal bars represent 3 min agonist applications. Increasing the potassium in the medium from 3 to 9 mM led to a small depolarization. In (a) the agonists were added to the cortical end of the LOT and in (b) to the cut (rostral) end of the LOT. 3-APS = 3-aminopropanesulphonic acid.

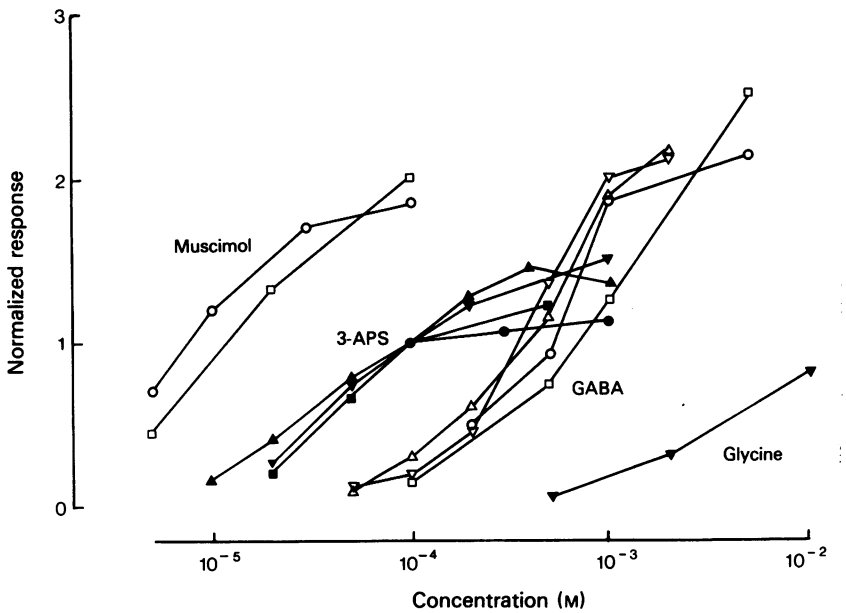

Figure 2 Dose-response curves to various agonists in 4 slices: each symbol represents the responses from a single slice. For display purposes the responses were normalized with respect to the response to 10^{-4} M 3-aminopropanesulphonic acid (3-APS) = 1.

The agents used were: GABA (Sigma), glycine (Koch-Light), L-glutamate (BDH), 3-aminopropanesulphonic acid (Alfred Bader Library of rare chemicals), carbachol (Sigma), muscimol (gift to Prof. D.W. Straughan from Dr C. Gardner, Roussel Labs.), taurine (Sigma), strychnine (Hopkin & Williams) and (+)-bicuculline (Sigma).

Results

Depolarizing responses to γ-aminobutyric acid

Exposure of the olfactory cortex slice to GABA caused a dose-dependent depolarization of the LOT (Figure 1). For 3 min applications, the threshold dose lay between 10^{-5} and 10^{-4} M GABA. The apparent maximum response was usually obtained with doses of between 5×10^{-3} M and 10^{-2} M GABA, but the response to these high doses often declined or 'faded' during the GABA application. Such responses were often followed by a shallow afterhyperpolarization (e.g. response to 2 mM GABA in Figure 1).

Although most of the present study was concerned with responses of the cortical end of the LOT, very similar responses could also be obtained from the cut, rostral end of the LOT by addition of the agonist to the other compartment of the bath (see Figure 1). Although the amplitude of the response to GABA varied widely from slice to slice, presumably due to

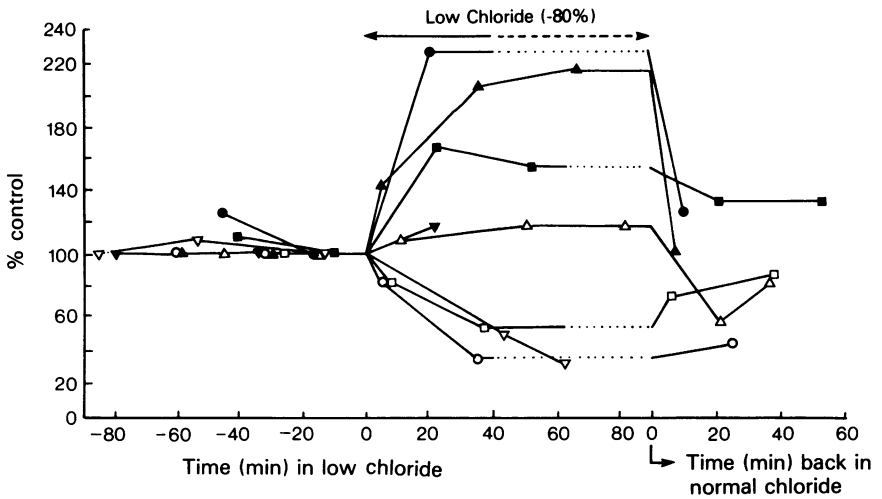

Figure 3 The effect of the replacement of 80% of the Cl^- in the medium by isethionate. Depolarizations to 9 mM K^+ (filled symbols) and to 5×10^{-4} M γ-aminobutyric acid (GABA, open symbols) are shown in 4 slices, expressed as % of control value. Three out of four GABA responses were reduced in low Cl^-, and all four K^+ responses were increased.

changes in the resistance of the barrier separating the two halves of the slice, the responses in individual slices to a standard GABA dose were consistent over many hours. There appeared to be little or no contribution of synaptic activity to the GABA responses since the presence of 20 mM Mg^{2+} left the responses unchanged or only slightly reduced. Similar responses to GABA could be obtained from slices maintained at 37°C. The tract could also be depolarized by raising the K^+ in the artificial c.s.f. medium from 3 to 9 mM.

Responses to other agonists

Muscimol was found to be the most potent depolarizing agent, with an apparent dose-ratio with GABA on the cortical end of the LOT of 68 (range 43 to 100 in 7 slices). This dose-ratio was only 20:1 (range 19 to 20 in 3 slices) on the rostral end of the LOT. At the lower response levels, 3-aminopropanesulphonic acid (3-APS) was also more potent than GABA (Figure 2) with an apparent dose-ratio with GABA of 5.6 (range 2.2 to 7.6 in 7 slices). However, the apparent maximum response to 3-APS was distinctly less than that to GABA.

Glycine and taurine were considerably less active and had shallower dose-response curves than did GABA. Comparison of the response evoked by 10^{-2} M glycine or taurine to the GABA dose-response curve gave apparent dose-ratios of 0.046 for glycine (range 0.014 to 0.15 in 21 slices) and 0.016 for taurine (range 0.006 to 0.036 in 4 slices). In contrast to its action in the superior cervical ganglion (Bowery & Brown, 1974), carbachol up to a concentration of 5×10^{-3} M was virtually ineffective in the LOT, as was glutamate up to a concentration of 5×10^{-3} M.

Low chloride medium

With extracellular recording methods it is difficult to test the ionic dependence of polarization changes. However, as GABA depolarizations elsewhere are said to be due to increased Cl^- conductance (Nishi, Minota & Karczmar, 1974), the effect was studied of replacing most of the Cl^- in the medium with the impermeant, but not necessarily inert anion, isethionate. As shown in Figure 3, the amplitude of the GABA-evoked depolarizations were depressed in low Cl^- medium while the depolarizations to K^+ were greatly enhanced.

γ-Aminobutyric acid antagonists

The actions of the GABA antagonists on the LOT responses were complicated, but probably no different from their actions on other GABA receptors. As seen in the superior cervical ganglion (Bowery & Brown, 1974) and on cuneate afferents (Simmonds, 1978), moderate doses (e.g. $>10^{-5}$ M) of both bicuculline and strychnine usually shifted the GABA dose-response curve to the right in an approximately parallel fashion. However, lower doses of antagonist could sometimes facilitate the GABA responses, especially with high GABA doses (Figure 4). Facilitation of

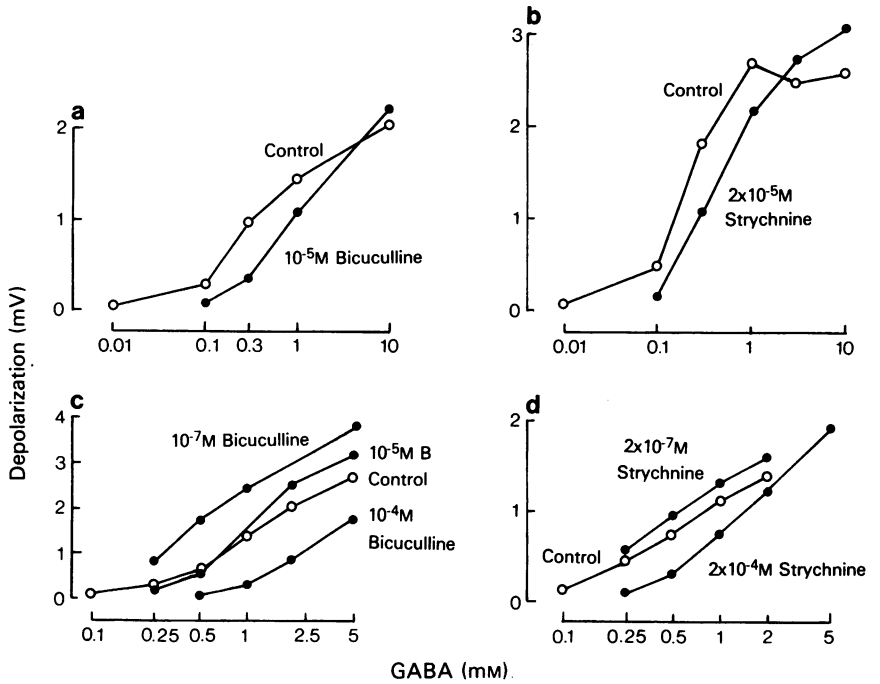

Figure 4 The effect of (+)-bicuculline (B) and of strychnine on the γ-aminobutyric acid (GABA) depolarizations in the lateral olfactory tract (LOT) in 4 slices. In (a) and (b) the lowest GABA doses were antagonized whereas the highest doses were facilitated. In (c) and (d) dose-response curves were shifted to the left by the lowest dose of antagonist, and shifted to the right at high doses. Each point represents a single response.

GABA responses by antagonists has previously been reported both *in vivo* (Hill, Simmonds & Straughan, 1973, Krnjević, Puil & Werman, 1977) and *in vitro* (Simmonds, 1978). By repeating a dose of GABA low enough to minimize any facilitatory effect, and using appropriate multiples of this dose in the presence of increasing concentrations of antagonist, estimates of pA_2s, pA_{10}s and IC_{50}s could be obtained. These values were: (+)-bicuculline, pA_2 5.00 (range 4.82 to 5.26 in 5 slices), pA_{10} 3.67 (range 3.10 to 4.00); IC_{50} 18 μM (range 6 to 34): strychnine, pA_2 4.27 (range 4.07 to 4.59), pA_{10} very low ($\ll 3$), IC_{50} 68 μM. These figures do not support the idea that bicuculline is a competitive GABA antagonist, but differential uptake rates could have distorted the values (Brown & Galvan, 1977).

In limited numbers of experiments, 3-APS and taurine appeared to be antagonized to a greater degree by bicuculline and strychnine than were equipotent doses of GABA, whereas the glycine depolarizations were antagonized less (Figure 5). Although postsynaptic hyperpolarizations to glycine are usually strychnine-sensitive, these findings are in agreement with the reported strychnine resistance of other glycine depolarizations (Oomura, Sawada, Tanikawa & Ooyama, 1974; Barker, Nicoll & Padjen, 1975).

Discussion

These experiments have confirmed that the lateral olfactory tract in the olfactory cortex can be depolarized by GABA. Since application of GABA to either end of the LOT produced equivalent depolarizations, it seems that GABA receptors may not be exclusive to nerve terminals but may be present all over the axonal membrane. Less likely alternative explanations for the recorded signal are electrogenic uptake (Kehoe, 1975), which could well apply to the glycine response, or glial responses (Cohen, 1970). Postsynaptic responses might have been recorded along centrifugal axons in the LOT (Heimer, 1968) although the lack of effect of glutamate, a potent depolarizing agent of neurones in the olfactory cortex (Richards, 1978), weighs against this.

At sites where intracellular recordings are possible, similar GABA depolarizations are a consequence of increased membrane conductance, especially to chlor-

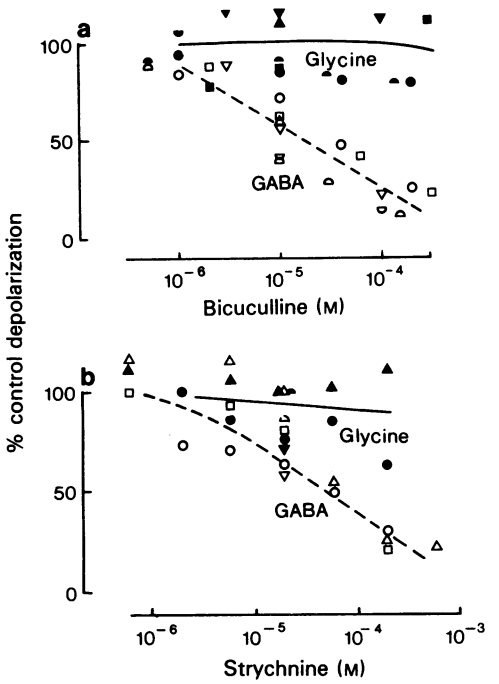

Figure 5 Effect of increasing concentrations of (a) bicuculline and (b) strychnine on lateral olfactory tract (LOT) depolarizations produced by 10 mM doses of glycine (filled symbols) and doses of γ-aminobutyric acid (GABA, $< 5 \times 10^{-4}$ M) low enough to minimize any facilitatory effects (open symbols). Each symbol pair represents a separate experiment and the lines were drawn by eye.

ide (Nishi *et al.*, 1974; Adams & Brown, 1975; Gallagher, Higashi & Nishi, 1978). Although a brief initial period of enhanced GABA responses might have been predicted for individual axonal responses in low chloride medium, this could well have been obscured in the rather asynchronous population responses of the present study. Nevertheless, the present results are still readily explicable in terms of Cl^--mediated depolarizations (see Nicoll, 1978; Brown & Marsh, 1978; Gallagher *et al.*, 1978). Enhanced K^+ responses in low Cl^- medium have been seen with comparable recording conditions in cuneate slices (Simmonds, 1978), which suggests that in these nerve tracts, as in muscle (Hodgkin & Horowicz, 1959), depolarizations to high K^+ are normally partially shunted through open Cl^- channels.

Compared to other *in vitro* preparations, relatively high doses of GABA were required to produce depolarizations in the olfactory cortex. However, the olfactory cortex was still covered by an intact pia which must act as a diffusional barrier, and uptake could play a more significant role in slices of cortex, as suggested by the exceptionally high muscimol:GABA dose-ratios.

As seen in several other preparations, the response to high doses of GABA (and also 3-APS and muscimol) 'faded' or declined during the application. Previously such 'fade' had been attributed, at least in part, to receptor desensitization (Adams & Brown, 1975). However, it is possible that there could be a contribution from a direct hyperpolarizing component in the GABA response, which is usually masked by the depolarization. Indeed, direct hyperpolarizations in response to GABA have occasionally been obtained in the LOT (unpublished observations) and have also been recorded in the superior cervical ganglion (Brown & Constanti, 1978). As had been noted previously (Simmonds, 1978), the paradoxical facilitation of the GABA depolarizations by bicuculline and strychnine can be associated with loss of 'fade'. This could be explained if 'fade' were due to an independent hyperpolarizing influence, which was antagonized preferentially to the depolarizations.

The action of the antagonists and the GABA analogues showed that the LOT GABA receptors are probably no different from those studied elsewhere (e.g. Bowery & Brown, 1974; Simmonds, 1978). The functional significance of the LOT receptors is not known; however, GABA receptors could be a normal constituent of excitable membranes. Although at some sites, such as at primary afferent terminals, such receptors may be utilized in presynaptic inhibition, at others such as peripheral nerve (Brown & Marsh, 1978), peripheral ganglia (de Groat, 1970), vascular (Fujiwara, Muramatsu & Shibata, 1975) and uterine muscle (Bedwani, Ishizawa, Pickles & Suwankrughasn, 1977) little exposure to GABA would be expected and the receptors may be vestigial, or have a different function that has not yet been appreciated. Presynaptic GABA receptors in the olfactory cortex may be different again, since in the cortex GABA is plentiful and is constantly released at postsynaptic inhibitory synapses, and sufficient diffusion might occur to depolarize presynaptic axons, at least under certain *in vitro* conditions (Pickles & Simmonds, 1976). It is not inconceivable that something similar could occur *in vivo* under conditions of massive GABA release or impaired removal of GABA.

Presynaptic GABA receptors could well be widespread in the CNS with GABA involved in non-synaptic transmitter actions (see Ramon-Moliner, 1977) or feedback regulation of its own release (Snodgrass, 1978). Even if the physiological function of GABA receptors such as those on the LOT is unknown, they could be important sites for drug action.

This work was supported by a grant from the M.R.C. to Dr M.A. Simmonds.

References

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

BARKER, J.L., NICOLL, R.A. & PADJEN A. (1975). Studies on convulsants in the isolated frog spinal cord. 1. Antagonism of amino acid responses. *J. Physiol.*, **245**, 521–536.

BEDWANI, J.R., ISHIZAWA, M., PICKLES, V.R. & SUWANKRUGHASN, S. (1977). Spasmogenic and potentiating actions of some amino acids on the guinea-pig myometrium. *Br. J. Pharmac.*, **61**, 217–222.

BOWERY, N.G. & BROWN, D.A. (1974). Depolarizing actions of γ-aminobutyric acid and related compounds on rat superior cervical ganglion *in vitro*. *Br. J. Pharmac.*, **50**, 205–218.

BROWN, D.A. & CONSTANTI, A. (1978). Interaction of pentobarbitone and γ-aminobutyric acid on mammalian sympathetic ganglion cells. *Br. J. Pharmac.*, **63**, 217–224.

BROWN, D.A. & GALVAN, M. (1977). Influence of neuroglial transport on the action of γ-aminobutyric acid on mammalian ganglion cells. *Br. J. Pharmac.*, **59**, 373–378.

BROWN, D.A. & MARSH, S. (1978). Axonal GABA-receptors in mammalian peripheral nerve trunks. *Brain Res.*, **156**, 187–191.

COHEN, M.W. (1970). The contribution of glial cells to surface recordings from the optic nerve of an amphibian. *J. Physiol.*, **210**, 565–580.

DE GROAT, W.C. (1970). The actions of γ-aminobutyric acid and related amino acids on mammalian autonomic ganglia. *J. Pharmac., exp., Ther.*, **172**, 384–396.

FUJIWARA, M., MURAMATSU, I. & SHIBATA, S. (1975). γ-Aminobutyric acid receptor on vascular smooth muscle of dog cerebral arteries. *Br. J. Pharmac.*, **55**, 561–562.

GALLAGHER, J.P., HIGASHI, H. & NISHI, S. (1978). Characterisation and ionic basis of GABA-induced depolarisations recorded *in vitro* from cat primary afferent neurons. *J. Physiol.*, **275**, 263–282.

HEIMER, L. (1968). Synaptic distribution of centripetal and centrifugal nerve fibres in the olfactory system of the rat. An experimental anatomical study. *J. Anat.*, **103**, 413–432.

HILL, R.G., SIMMONDS, M.A. & STRAUGHAN, D.W. (1973). A comparative study of some convulsant substances as γ-aminobutyric acid antagonists in the feline cerebral cortex. *Br. J. Pharmac.*, **49**, 37–51.

HODGKIN, A.L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol.*, **148**, 127–160.

KEHOE, J. (1976). Electrogenic effects of neutral amino acids on neurons of *Aplysia Californica. Cold Spring Harbor Symp. quant. Biol.*, **XL**, 145–155.

KRNJEVIĆ, K. PUIL, E. & WERMAN, R. (1977). Bicuculline, benzyl penicillin and inhibitory amino acids in the spinal cord of the cat. *Can. J. Physiol., Pharmac.*, **55**, 670–680.

LEVY, R.A. (1974). GABA: a direct depolarising action at the mammalian primary afferent terminal. *Brain Res.*, **76**, 155–160.

NICOLL, R.A. (1978). Physiological studies on amino acids and peptides as prospective transmitters in the CNS. In *Psychopharmacology: A Generation of Progress.* ed. Lipton M.A., DiMascio, A. & Killam, K.F. pp. 103–118. New York: Raven Press.

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

OOMURA, Y., SAWADA, M., TANIKAWA, I. & OOYAMA, H. (1974). Depolarization of *Onchidium* neurone by glycine. *Nature*, **250**, 258–260.

PICKLES, H.G. & SIMMONDS, M.A. (1976). Possible presynaptic inhibition in rat olfactory cortex. *J. Physiol.*, **260**, 475–486.

RAMON-MOLINER, E. (1977). Non-synaptic chemical neurotransmission. *Experientia*, **33**, 1342–1344.

RICHARDS, C.D. (1978). Evidence of localization of glutamate receptors in layer 1A of the dendritic field of neurones in the prepiriform cortex. In *Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System.* ed. Ryall, R.W. & Kelly, J.S. pp. 185–187. Amsterdam: Elsevier.

SNODGRASS, S.R. (1978). Use of ^{3}H-muscimol for GABA receptor studies. *Nature*, **273**, 392–394.

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.

SIMMONDS, M.A., & PICKLES, H.G. (1978). Presynaptic actions of GABA in isolated slices of cuneate nucleus and olfactory cortex. In *Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System.* ed. Ryall, R.W. & Kelly, J.S., pp. 279–281. Amsterdam: Elsevier.

(Received April 24, 1978
Revised August 22, 1978.)