Br. J. Pharmac. (1984), **81**, 327–333

Coexistence of $GABA_A$ and $GABA_B$ receptors on Aδ and C primary afferents

Michel Désarmenien, Paul Feltz, Guiseppe Occhipinti[1], Francesca Santangelo[1] & Remy Schlichter

Institut de Physiologie et de Chimie Biologique, 21 rue René Descartes, 67084 Strasbourg, Cedex, Université Louis Pasteur, LA 309 CNRS, France

1 Intracellular recordings from adult rat dorsal root ganglion neurones were performed *in vitro* and the coexistence of two γ-aminobutyric acid (GABA) receptors on the membrane of identified Aδ and C primary afferents was demonstrated.

2 Transient applications of GABA ($10^{-6} - 10^{-2}$ M) evoked dose-dependent depolarizations and increased membrane conductance. The responses were mimicked by muscimol, isoguvacine, THIP and 3 amino propane sulphonic acid (3 APS); they were blocked by bicuculline and picrotoxin. Pentobarbitone induced an increase of GABA-induced depolarizations.

3 Perfusion of tetraethylammonium (TEA, 7.5 mM) and intracellular injection of Cs^+ ions unmasked the Ca^{2+} component of action potentials, which appeared as long-lasting plateau depolarizations. Such action potentials were shortened in the presence of methoxyverapamil (D600, $5 \times 10^{-6} - 10^{-5}$ M) and in a medium without Ca^+ ions.

4 Prolonged (5–10 min) perfusion of GABA ($10^{-9} - 10^{-5}$ M) shortened the Ca^{2+} component of action potentials. This effect was mimicked by baclofen ($10^{-7} - 5 \times 10^{-6}$ M) and muscimol ($5 \times 10^{-7} - 10^{-5}$ M) and was not affected by bicuculline perfusion ($5 \times 10^{-6} - 10^{-5}$ M). Isoguvacine (2.5×10^{-5} M) did not affect action potential duration.

5 It is concluded that two GABA receptors coexist on the membrane of slow conducting primary afferents: the bicuculline-sensitive $GABA_A$ receptor mediates depolarizations and the bicuculline-insensitive $GABA_B$ receptor shortens the calcium component of action potentials.

Introduction

Classically, γ-aminobutyric acid (GABA)-induced presynaptic inhibitions have been related to the depolarizing action of GABA, monitored in the spinal cord by means of either excitability tests (for references see Curtis & Lodge, 1982 and Rudomin & Jankowska, 1982) or intraaxonal recordings from large primary afferents (Jankowska *et al.* (1982). However, a recent study performed on sympathetic ganglia showed that the GABA-induced decrease of acetylcholine release was insensitive to bicuculline, which blocked GABA-induced depolarizations (Brown & Higgins, 1979) and pharmacological discrepancies have been described between dorsal root depolarizations and the depressant action of GABA-related compounds on synaptic activity (Allan *et al.*, 1980). Recently, a bicuculline-insensitive GABA receptor ($GABA_B$) has been described (Hill & Bowery, 1981), the activation of which decreases transmitter release in various central and peripheral preparations. Thus, GABA-ergic presynaptic inhibitions seem to be mediated by at least two pharmacologically distinct receptors: $GABA_A$ and $GABA_B$. The $GABA_A$ receptor is selectively activated by isoguvacine and specifically blocked by bicuculline, the $GABA_B$ receptor is selectively activated by (−)-baclofen and insensitive to bicuculline. Electrophysiological studies on dorsal root ganglion cells indicate that the activation of $GABA_A$ receptors induces a chloride-dependant depolarization of sensory neurones (see Gallagher *et al.*, 1978; Désarmenien *et al.*, 1980). In cultured chick dorsal root ganglion neurones, $GABA_B$ receptors activation can be monitored as a shortening of the calcium component of action potentials (Dunlap, 1981).

To study these two types of GABA action we have

[1]Present address: Instituto di Fisiologia Umana, Via A. Doria 6, 95125 Catania, Italy.

made intracellular recordings from various types of dorsal root ganglion (DRG) neurones and have demonstrated the coexistence of $GABA_A$ and $GABA_B$ receptors on both Aδ and C primary afferents. These slow conducting afferents were chosen because: (1) Aδ and C DRG cells are depolarized by GABA, although to a lesser extent than are large fast conducting neurones (Désarmenien, *et al.*, 1981b). (2) GABA depolarizes unmyelinated sensory fibres (Brown & Marsh, 1978) and a spinal presynaptic inhibition of C fibres has been demonstrated (Calvillo *et al*, 1982); (3) they often display action potentials with a calcium component (Yoshida & Matsuda, 1979), a prerequisite for the mode of electrophysiological detection of $GABA_B$ receptors selected. A preliminary account of this work has been published (Désarmenien *et al.*, 1982).

Methods

Lumbar dorsal root ganglia (L_4–L_5) were dissected out from Charles River rats (4–8 weeks old) and one selected ganglion was placed in a superfusion chamber on the stage of a microscope, following the procedure already described (Désarmenien *et al.*, 1981a). Under visual control (magnification × 320), small neurones were selected and impaled with glass microelectrodes (tip resistance 50–150 MΩ) containing K-acetate (4 M), CsCl or Cs_2SO_4 (2 M). Anti- or ortho-dromic action potentials were evoked at two different distances from the ganglion and conduction velocity was calculated from the difference between their latencies (see details in Désarmenien *et al.*, 1983). Hyperpolarizing current pulses (0.05–1nA; 60 ms at 1 Hz) were applied to monitor membrane resistance via a conventional bridge circuit.

The ganglion was continuously superfused with Ringer solution of the following composition (mM): NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 25 and glucose 11. The solution was maintained at 30–35°C and bubbled with 95% O_2 and 5%CO_2 (pH 7.4). A rate of flow of 1–3 ml min^{-1} allowed rapid exchanges in the chamber (total volume: 0.25 ml). Ca^{2+}-free solutions were obtained by substitution of $CaCl_2$ with $MgCl_2$. Baclofen was prepared from a stock solution at 10^{-2} M (diluted in HCl 0.1 N; pH adjusted to 3 with NaOH).

Drugs were applied by continuous or transient perfusion: 5 µl drops of calibrated solutions placed directly in the perfusion stream (see details in Désarmenien *et al.*, 1981a). The drugs used in this study were: GABA (SIGMA and N & B Co), muscimol (gift from Synthelabo Paris), 3 amino propane sulphonic acid (3 APS, K and K laboratories) isoguvacine (gift from Dr Krogsgaard Larsen), bicuculline (Pierce and Synthelabo), picrotoxin (Sigma), pentobarbitone (May & Baker), (±)-β-*p*-chlorophenyl-GABA (baclofen, Ciba-Geigy), (−)-baclofen (Ciba-Geigy), methoxyverapamil hydrochloride (D600, Knoll) and tetraethylammonium Cl (TEA-Merck and Flucka).

Results

The results presented here were obtained from 121 small surface neurones (mean diameter 31.4 ± 1.8 µm; $n = 40$). Only cells with a conduction velocity slower than 15 ms^{-1} were considered, except for 8 unidentified neurones on which the calcium component of action potentials was studied. Neurones with a conduction velocity slower than 2.1 m s^{-1} were classified as C cells and those with a conduction velocity between 2.5 and 15 m s^{-1} were considered as Aδ; some of their characteristics are indicated in Table 1.

GABA-induced depolarizations

Drop applications of GABA elicited transient depolarizations and decreased membrane resistance in all 11 Aδ and 29 C cells tested. The amplitude of the responses was dose-dependent within the range 10^{-6}–10^{-2} M, the maximal amplitude of the response being 10.8 ± 1 mV in the C cells and 15.7 ± 2.5 mV in the Aδ cells. Successive applications produced progressively smaller responses, similar to what we have

Table 1 Electrophysiological characteristics of Aδ and C dorsal root ganglion cells

	V cond. (ms^{-1})	*RP* (mV)	*IR* (MΩ)	*Diameter* (µm)
C cells	0.74 ± 0.05 $n = 56$	64 ± 1.8 $n = 56$	49 ± 7 $n = 41$	25 ± 1.2 $n = 24$
Aδ cells	8.9 ± 0.5 $n = 65$	64 ± 1.5 $n = 63$	38 ± 4 $n = 36$	40 ± 3 $n = 16$

V cond. = conduction velocity; RP = resting membrane potential; IR = input resistance. Numbers (n) indicate the number of cells and the values are indicated ± s.e.mean.

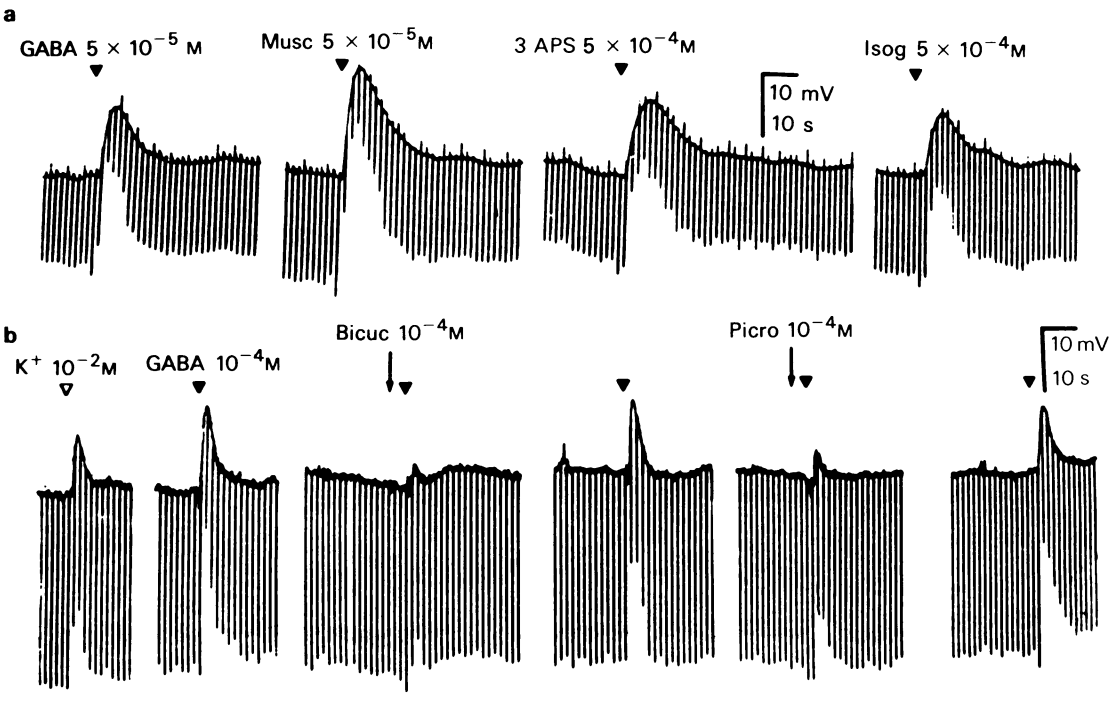

Figure 1 Pharmacological characteristics of the $GABA_A$ receptor. (a) GABA-induced depolarization and decrease in membrane conductance are mimicked by a series of agonists. Note that different concentrations lead to comparable responses, illustrating the relative potency of the various agonists. Transient hyperpolarizations (1 nA, 60 ms) monitor the membrane resistance. C cell (V cond = 0.6 m s^{-1}) resting potential − 75 mV, membrane resistance 13 MΩ. (b) GABA-induced responses are reversibly reduced by a previous application of bicuculline (Bicuc, middle) or picrotoxin (Picro, right). Note that GABA and the antagonists were applied transiently by drop (see methods), this explains the relatively high concentrations used. Transient applications of KCl (left) monitor the dilution of the drops in the bath, used to calculate the concentration attained over the cell. C cells (V cond = 0.4 m s^{-1}) resting potential −85 mV, membrane resistance 230 MΩ.
Musc = muscimol; 3 APS = 3 amino propane sulphonic acid; Isog = isoguvacine.

described in large neurones as due to a desensitization process. This property of $GABA_A$ receptors was used to perform cross-desensitization tests between GABA and all agonists tested.

As shown in Figure 1, the responses were mimicked by muscimol (15 C and 5 Aδ cells), 3 APS (11 C and 5Aδ cells) and isoguvacine (10 C and 5 Aδ cells), which were respectively 3 times, 0.45 times and 0.3 times as potent as GABA. Bicuculline (10^{-5}–10^{-4}M, 8 C and 5 Aδ cells) and picrotoxin (10^{-5}–10^{-3}M, 6C and 3 Aδ cells) applied by drop 5 s before GABA (10^{-5}–10^{-4}M), decreased the depolarizations by 66 ± 11% and 71 ± 30% respectively (Figure 1). Pentobarbitone applied similarly, enhanced the depolarizations obtained with low doses of GABA (5×10^{-5}M) by 71.5 ± 20% in 8 C and 2 Aδ cells.

The calcium component of action potentials

The duration of action potentials was measured as the time during which they decayed from their maximal value to half this maximum, also called time to half decay and referred to as t.h.d.

Perfusion of tetraethylammonium (TEA, 7.5–10 mM) increased the t.h.d. up to 70 ms in 17 C and 6 Aδ cells (initial spike duration 3.4 ± 0.3 and 1.3 ± 0.2 ms respectively).

Injection of Cs^+ ions from the recording micropipette, by means of depolarizing current pulses (0.1–1 nA, 60 ms at 1 Hz) progressively increased the t.h.d. up to 230 ms in 10 C cells (initial t.h.d.: 4.5 ± 0.8 ms). The study of prolonged action potentials was routinely performed by adding TEA (7.5 mM) to the medium at the beginning of the experiments and starting Cs^+ injection immediately after cell impalement. Under these conditions, the action potential (26 ± 11 ms at impalement, $n = 49$) was progressively prolonged in a long-lasting depolarization (1.7 ± 1.4 s, $n = 42$) as shown in Figure 3.

Substitution of $CaCl_2$ by $MgCl_2$ induced a reversi-

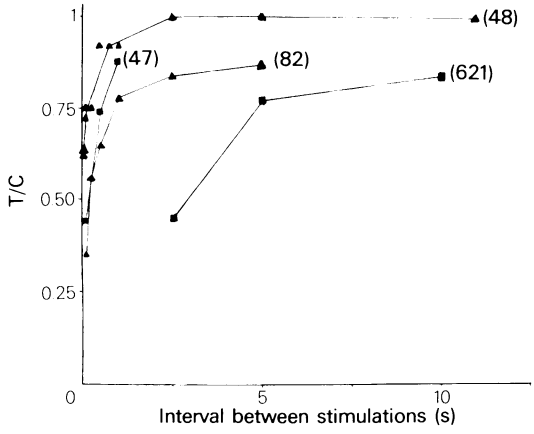

Figure 2 Action potential duration is reduced when the stimulation frequency is increased in rat dorsal root ganglion neurones. In four different cells, a control (C) action potential was evoked every 2 mn and followed by a test (T) action potential at varying intervals. Their relative duration (T/C) was plotted against the stimulation interval. Numbers in parentheses indicate the duration of the control action potential (ms).

ble shortening of prolonged action potentials by $70 \pm 5\%$ in 11 cells, and further addition of $BaCl_2$ (5 mM) in this medium restored a similar or longer plateau phase in 3 cells. Perfusion of D600 (5×10^{-6}–10^{-5} M) reversibly decreased the t.h.d. by $91 \pm 6\%$ in 6 cells.

Stable action potential duration was a prerequisite for the study of GABA actions on their calcium component and this could be achieved satisfactorily only after control of the membrane potential (by manually controlled d.c. current injection) and stimulation rate (maintained under 0.01–0.05 Hz). The voltage-dependency of the spikes t.h.d. was determined in 7 cells and a dramatic increase occurred with depolarization (t.h.d. from 3 to 70 ms when the membrane potential was varied from −60 to −10 mV). The sensitivity of spike t.h.d. to the stimulation rate was determined in 10 cells, using paired stimulations (50–0.05 s stimulus-interval) applied each 30 s at least. A pronounced decrease of spike t.h.d. (up to 70%) was observed with a spike-interval shorter than 5 s (Figure 2).

$GABA_B$ receptor activation

As shown in Figure 3, continuous perfusion of GABA (10^{-9}–10^{-5} M; 5–16 min) reduced the t.h.d. in 16 cells by $23.3 \pm 3.6\%$ (7 C cells and 9 Aδ cells). This effect, which appeared progressively within 3–5 min, was reversible after washing and showed no desensitization during prolonged perfusion; it appeared with doses which affected neither the resting potential nor the membrane resistance. Muscimol (5×10^{-7}–10^{-5} M) decreased the t.h.d. by $50 \pm 5\%$ in all cells tested. The effect was also mimicked by (±)-baclofen and by (−)-baclofen (10^{-7}–10^{-5} M; 3–15 min) which reduced the t.h.d. by $32 \pm 6\%$ in 20 cells (17 Aδ and 3C). In a large number of cells, excess Cs^+ loading produced a continuous prolongation of action potentials and satisfactory measurements of agonists effects on the t.h.d. could not be made. However, we noticed that in these cells, GABA and baclofen induced no marked reduction of action potential t.h.d. Thus, 15 cells (2 with GABA and 13 with baclofen) were recorded with K acetate (4 M) in the pipette instead of CsCl and agonists reduced the t.h.d. in all these 15 cells. Bicuculline alone generally prolonged the t.h.d. (5×10^{-6}–10^{-5} M; 4 cells) by 100 to 140%, except in one cell in which the t.h.d. was reduced by 45% during bicuculline (10^{-5} M) perfusion. Input membrane potential and resistance were unaffected by bicuculline (8 cells). Bicuculline (10^{-6}–10^{-5} M) was applied during perfusion of GABA (10^{-7}, 10^{-5} M; 2 cells), baclofen (10^{-6}–10^{-5} M; 9 cells), and muscimol (10^{-5} M; 1 cell) and had no effect on the t.h.d. of reduced action potentials. Isoguvacine (10^{-6}–2.5×10^{-4} M) did not affect the t.h.d. in the three cells tested.

Discussion

Our results lead to the conclusion that $GABA_A$ and $GABA_B$ receptors coexist on Aδ and C primary afferent neurones. The clear similarities between the pharmacological properties of GABA-induced depolarizations on the various classes of primary afferents indicate a homogeneous class of $GABA_A$ receptors, even though the ionic mechanisms they trigger could differ somewhat from cell to cell (see discussion in Désarmenien *et al.*, 1983). On the other hand, the shortening by GABA of the calcium component of action potentials described here is probably due to the activation of a receptor similar to the binding site described by Bowery *et al.*, (1981). Indeed, this site binds baclofen (which is not active on $GABA_A$ receptors, unpublished observations, and see Ault & Evans, 1978) and muscimol (although with a lower affinity) but not bicuculline, isoguvacine or THIP. Furthermore, it has a higher affinity for GABA than the $GABA_A$ site, and we observed that GABA shortens action potentials at doses ($< 10^{-6}$ M) at which it does not affect membrane potential and resistance. A comparison of dose-response relationships for $GABA_A$ and $GABA_B$ effects could not be obtained satisfactorily in DRG cells mainly because of the fast desensitization of $GABA_A$ receptors, which introduce unmeasurable underestimations of the depolar-

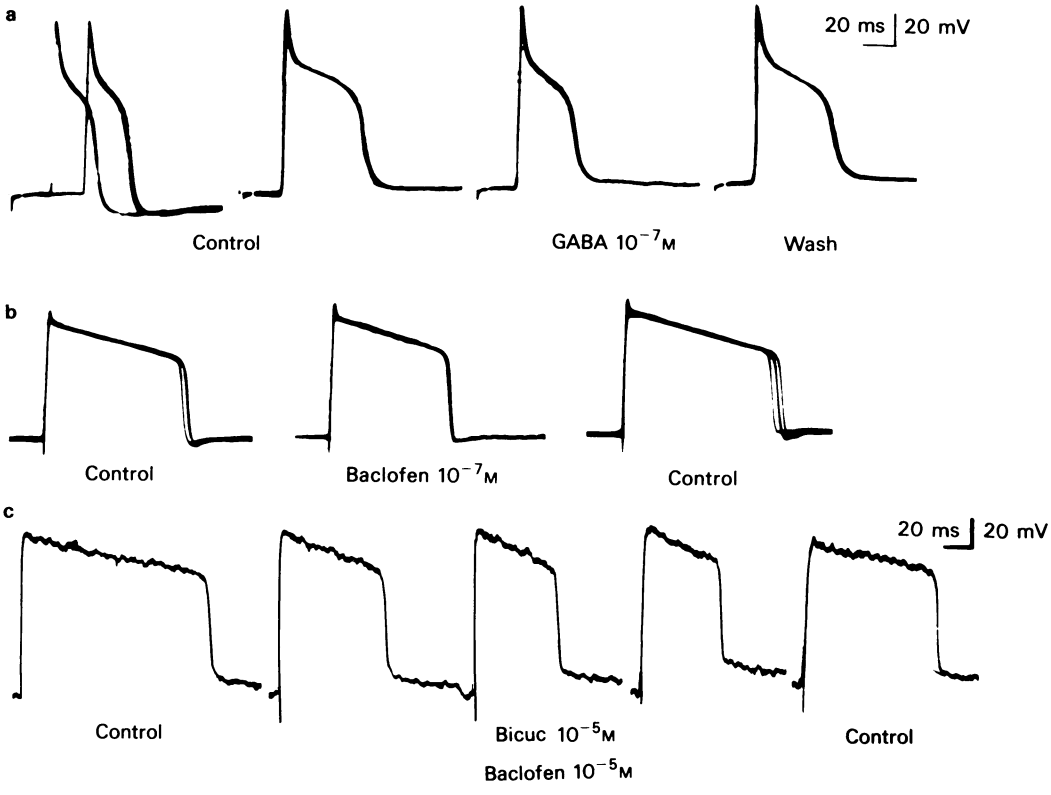

Figure 3 Reduction by $GABA_B$ agonists of the calcium component of action potentials in dorsal root ganglion cells. (a) Left, antidromic action potential evoked from two distances from the ganglion and recorded in a C cell (conduction velocity: 0.5 m s^{-1}). Right, superimposed action potentials recorded in a control medium, during GABA perfusion and after washing, illustrating GABA-induced shortening of the calcium component of action potentials. (b) Superimposed action potentials recorded in an Aδ cell before, during and after perfusion of (±)-baclofen, which had a similar action to that of GABA. Same calibrations as in top row. (c) Action potentials recorded in an Aδ cell illustrating the bicuculline-insensitivity of baclofen action. The following perfusion sequence was applied: Control Ringer, baclofen (10^{-5}M), baclofen (10^{-5}M) + bicuculline (10^{-5}M),baclofen, and control Ringer.
TEA (7.5 mM in the medium) and Cs^+ (injected intracellularly) were present throughout these experiments.

ization and conductance changes; and also because of technical difficulties (recording from small cells were made for less than 1 h, precluding the application of more than two doses of $GABA_B$ agonists). It is noteworthy that the shortening of the calcium component of action potentials described here (and in Dunlap, 1981) could explain how $GABA_B$ receptor activation decreases transmitter release at peripheral and central synapses (Bowery *et al.*, 1980; 1981).

The mechanism of this action, probably involving an intracellular messenger from the $GABA_B$ receptor to the ionic channels involved, remains to be determined. One hypothesis to explain the GABA effect on the action potential could be a reduction of calcium currents, as suggested by Dunlap (1981); GABA would thus act like noradrenaline in the sympathetic ganglion (Galvan & Adams, 1982). On the other hand, an involvement of potassium permeability cannot be excluded, since potassium channels of vertebrate neurones are not all blocked by TEA (Adams *et al.*, 1982). GABA would in this case increase K^+ permeability, in opposition to 5-hydroxytryptamine which decreases K^+ permeability, via an increase of intracellular cyclic AMP (Siegelbaum *et al.*, 1982), in invertebrate neurones. GABA could open or facilitate the opening of K^+ channels activatable at depolarized potentials only (e.g. during the plateau phase of action potentials); this hypothesis is supported by the fact that we did not observe GABA or baclofen-induced shortening of action potentials in cells excessively loaded with Cs^+ ions.

In some peripheral preparations, it seems clear that the $GABA_B$ receptor is the main factor modulating transmitter release (see Brown & Higgins, 1979; Bowery *et al.*, 1981) . The relative role of $GABA_A$ and $GABA_B$ receptors is not so far established in the spinal cord, like their relative distribution on the various classes of primary afferents. As far as large afferents are concerned, the presence of a $GABA_A$ receptor is well documented (see Curtis & Lodge, 1982) and its functional role is supported at least by the sensitivity of presynaptic inhibition to bicuculline (Curtis *et al*, 1971). Although we could not detect a $GABA_B$ receptor on these fibres, this is probably due to the fact that they displayed action potentials with little, if any, calcium component even in the presence of TEA and Cs^+ (but see Kostyuk *et al.*, 1981); indeed, the fact that low concentrations of baclofen produced a presynaptic inhibition of motoneuronal excitatory postsynaptic potentials (Fox *et al.*, 1978) indicate that large afferents probably possess a functional $GABA_B$ receptor.

Concerning the C fibres, it has been reported recently that they are the target of a presynaptic inhibition, monitored as an increase of terminal excitability (Calvillo *et al.*, 1982). This suggests that the $GABA_A$ receptor which induces depolarizations of C fibres (Brown & Marsh, 1978) and ganglion cells (Désarmenien *et al.*, 1981b and this paper), is functionally involved in presynaptic inhibition of these afferents. By extrapolation of the results presented here, it can be postulated that $GABA_B$ receptors are present on C afferent terminals, even though their functional role in presynaptic inhibition remains to be demonstrated.

As far as we know, Aδ cells have not yet been studied for the presence of GABA receptors by other authors. By extrapolation of the results presented here, it can be postulated that Aδ afferent terminals may possess $GABA_A$ and $GABA_B$ receptors.

The respective role of $GABA_A$ and $GABA_B$ receptors in spinal presynaptic inhibition and in the modulation of transmitter release remains unclear. A large number of questions concerning the physiological role of $GABA_B$ receptors will be answered only after a selective and potent inhibitor for $GABA_B$ actions is found. This antagonist should be as reliable as bicuculline, which is the final support for demonstration of the physiological role of $GABA_A$ receptors, whether they have a post- or a presynaptic location.

This study was supported by INSERM and CNRS (PRC 121009/ATP 2581).

References

ADAMS, P., CONSTANTI, A., BROWN, D.A. & CLARK, R.B. (1982). Intracellular Ca^{++} activates a fast voltage-sensitive K^+ current in vertebrate sympathetic neurons. *Nature*, **296,** 746–749.

ALLAN, R.D., EVANS, R.H. & JOHNSTON, G.A.R. (1980). γ aminobutyric acid agonists: an in vitro comparison between depression of spinal synaptic activity and depolarization of spinal root fibres in the rat. *Br. J. Pharmac.*, **70,** 609–615.

AULT, B. & EVANS, R.H. (1978). Central depressant action of baclofen. *J. Physiol.* **284,** 131P.

BOWERY, N.G., HILL, D.R., HUDSON, A.L., DOBLE, A., MIDDLEMISS, D.N., SHAW, J. & TURNBULL, M. (1980). (−) Baclofen decreases neurotransmitter release in the mammalian C.N.S. by an action at a novel GABA receptor. *Nature.*, **283,** 92–94.

BOWERY, N.G., DOBLE, A., HILL, D.R., HUDSON, A.L., SHAW, S.S., TURNBULL, M.J. & WARRINGTON, R. (1981). Bicuculline insensitive GABA receptors on peripheral autonomic nerve terminals. *Eur. J. Pharmac.*, **71,** 53–70.

BROWN, D.A. & HIGGINS, A.J. (1979). Presynaptic effects of GABA in isolated rat superior cervical ganglia. *Br. J. Pharmac.*, **66,** 108P.

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

CALVILLO, O., MADRID, J. & RUDOMIN, P. (1982). Presynaptic depolarization of unmyelinated primary afferent fibres in the spinal cord of the cat. *Neuroscience.*, **7,** 1389–1400.

CURTIS, D.R., DUGGAN, A.W., FELIX, D. & JOHNSTON, G.A.R. (1971). Bicuculline an agonist of GABA and synaptic inhibition in the spinal cord of the cat. *Brain Res.*, **32,** 69–96.

CURTIS, D.R., & LODGE, D. (1982). The depolarization of feline ventral horn group I_A spinal afferent terminations by GABA. *Exp. Brain Res.*, **46,** 215–233.

DÉSARMENIEN, M., FELTZ, P. & HEADLEY, P.M. (1980). Does glial uptake affect GABA responses? An intracellular study on rat dorsal root ganglion neurons in vitro. *J. Physiol.*, **307,** 163–182.

DÉSARMENIEN, M., FELTZ, P., HEADLEY, P.M. & SANTANGELO, F. (1981a). SL 75102 as a GABA agonist: experiments on dorsal root ganglion neurones *in vitro*. *Br. J. Pharmac.*, **72,** 355–364.

DÉSARMENIEN, M., FELTZ, P., LOEFFLER, J.H., OCCHIPINTI, G. SANTANGELO, F. (1982). Multiple GABA receptors on Aδ and C primary afferent neurones in the adult rat. *Br. J. Pharmac.*, **76,** 289P

DÉSARMENIEN, M., SANTANGELO, F., LINCK, G., HEADLEY, P.M. & FELTZ, P. (1981b). Physiological study of amino-acid uptake and receptor desensitization: the GABA system in dorsal root ganglia. In *Amino-acid Neurotransmitters.* ed. De Feudis, F.V. & Mandel, P. *Adv. Biochem. Psychophamac.*, Vol. 37, pp. 309–319. New York: Raven Press.

DÉSARMENIEN, M., SANTANGELO, F., LOEFFLER, J.PH. &

FELTZ, P. (1983). Comparative study of GABA-mediated depolarisations of lumbar Aδ and C primary afferent neurones of the rat. *Exp. Brain Res.*, (in press).

DUNLAP, K. (1981). Two types of γ-aminobutyric acid receptors on embryonic sensory neurones. *Br. J. Pharmac.*, **66,** 579–585.

FOX, S., KRNJEVIC, K., MORRIS, M.E., PUIL, E. & WERMAN, R. (1978). Action of Baclofen on mammalian synaptic transmission. *Neuroscience*, **3,** 495–515.

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

GALVAN, M. & ADAMS, P.R. (1982). Control of Calcium current in rat sympathetic neurons by norepinephrine. *Brain Res.*, **244,** 135–144.

HILL, D.R. & BOWERY, N.G. (1981). ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline – insensitive $GABA_B$ sites in rat brain. *Nature*, **290,** 149–152.

JANKOWSKA, E., McCREA, D., RUDOMIN, P. & SYKOVA, E. (1982). Observations on neuronal pathways subserving primary afferent depolarization. *J. Neurophysiol.*, **46,** 506–516.

KOSTYUK, P.G., VESELOVSKY, V.S. & FEDULOVA, S.A. (1981). Ionic currents in the somatic membrane of rat dorsal root ganglion neurons. II. Calcium currents. *Neurosc. Letters*, **6,** 2432–2437.

RUDOMIN, P. & JANKOVSKA, E. (1982). Presynaptic depolarization of rubro-spinal tract fibres in intermediate nucleus of cat spinal cord. *J. Neurophysiol.*, **46,** 517–531.

SIEGELBAUM, S.A., CAMARDO, J.S., & KANDEL, E.R. (1982). Serotonin and cyclic AMP close single K^+ channels in Aplysia sensory neurones. *Nature*, **299,** 413–417.

YOSHIDA, S. & MATSUDA, M. (1979). Studies on sensory neurones of the mouse with intracellular recording and horseradish peroxidase – injection techniques. *J. Neurophysiol.*, **42,** 1134–1145.

(Received August 4, 1983.
Revised October 10, 1983)