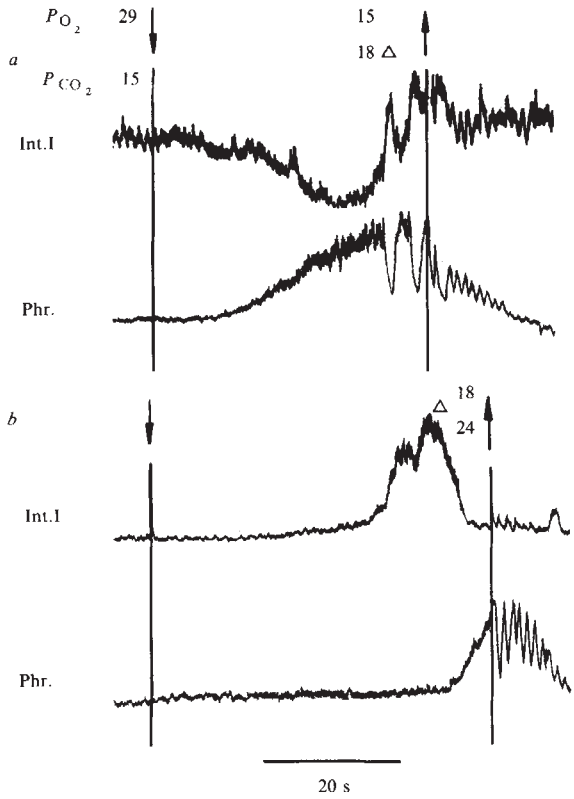

Fig. 3 Response of internal intercostal and phrenic motoneurones to stopping the respiratory pump, starting with steady-state hypoxic hypocapnic apnoea. *a*, Control; *b*, post-denervation of arterial peripheral chemoreceptors. Traces are integrated records for Int. I (T. 11) discharge and C5 Phr. neurogram. Arterial blood gases in torr (mm Hg). The pump was stopped at the downward arrow and restarted at the upward arrow. Abbreviations are the same as in Fig. 1.

inhibition must clearly have an important role in determining the overall balance of inspiratory and expiratory motoneurone discharge.

What can be said about the relation of these tonic activities to the generation of periodic activity? Figure 3*a* shows that with hypoxic hypocapnia, the occurrence of rhythm, due to the combined effects of increasing CO_2 and hypoxia, is expressed as a phasic burst of expiratory activity reciprocal to the phasic inhibition of inspiratory motoneurones. Yet previously it had been shown that in hypocapnic apnoea the occurrence of rhythm is expressed as a phasic inhibition of tonic expiratory activity. However, as seen in Fig. 3, the peak of the expiratory burst before peripheral chemoreceptor denervation (*a*) reaches the same tonic discharge level that after denervation (*b*) is due only to the central action of CO_2. Thus, before rhythm started the excitatory synaptic drive to the expiratory motoneurones coexisted with the drive to the inspiratory motoneurones, each counteracted through mutual reciprocal inhibition. The role of the rhythm generator can then be understood as a mechanism which we propose alternately inhibits the inspiratory and expiratory bulbospinal neurones, thus allowing the disinhibited pathway to express the intensity and balance of the prevailing chemical drives.

This work was supported by the Brain Research Trust, a Guggenheim Fellowship to A. J. B., and a Josiah Macy Fellowship to E.A.P.

Received 27 May; accepted 28 July 1982.

1. Sears, T. A. *Fedn Proc.* **36,** 2412–2420 (1977).
2. Bainton, C. R., Kirkwood, P. A. & Sears, T. A. *J. Physiol., Lond.* **280,** 249–272 (1978).
3. Bainton, C. R. & Kirkwood, P. A. *J. Physiol., Lond.* **296,** 291–314 (1979).
4. Sears, T. A. & Stagg, D. *J. Physiol., Lond.* **263,** 357–381 (1976).
5. Sears, T. A. *J. Physiol., Lond.* **175,** 404–424 (1964).
6. Berger, A. J. *J. Neurophysiol.* **42,** 76–90 (1979).

Heterogeneous sensitivity of cultured dorsal root ganglion neurones to opioid peptides selective for μ- and δ-opiate receptors

Mary Ann Werz* & Robert L. Macdonald†

* Neurosciences Program and †Department of Neurology, University of Michigan Medical Center, Neuroscience Laboratory Building, 1103 E Huron, Ann Arbor, Michigan 48109, USA

Opiate-mediated analgesia at the spinal level is thought to involve opiates binding to opiate receptors on primary afferent terminals[1–3] resulting in a selective depression of neurotransmitter release[4–9]. Multiple opiate receptor types have been distinguished[11–22] and μ- and δ-opiate receptors, originally described by Kosterlitz *et al.*[17,18,22], have been demonstrated on primary afferent terminals[1] but the correspondence of these opiate receptors to opiate-mediated depression of transmitter release is unclear. However, opiates binding to receptors present on individual somata of the dorsal root ganglion (DRG) neurones in dissociated cell culture have been reported to reduce the duration and amplitude of calcium-dependent action potentials[8,10]. Therefore these opiate receptors might have a function similar to those on primary afferent terminals where a decrease in calcium entry would be correlated with a decrease in transmitter release[23,24]. We have now studied the response of DRG neurones to opiate agonists with different affinity for μ- or δ-receptors and our results suggest that both receptor types can mediate decrease in somatic calcium-dependent action potentials but that there is a variable proportion of μ- and δ-receptors on DRG neurones.

For these studies we selected the opioid peptides morphiceptin and leucine-enkephalin. Leucine-enkephalin (Leu-enkephalin) is about 1,000-fold more potent than morphiceptin at δ-receptors whereas the two ligands are approximately equipotent at μ-receptors[11,17,18,22,29]. Preparation of mouse spinal cord and DRG co-cultures and electrophysiological methods were as previously described[28]. DRG neurones were impaled with 4 M potassium acetate-filled micropipettes (20–40 MΩ), and somatic calcium-dependent action potentials were evoked from resting membrane potential with 100-μs depolarizing current pulses at a frequency of 4 per min. Action potential durations were generally between 7 and 20 ms due to the addition of 5 mM calcium and 5 mM tetraethylammonium chloride (TEA) to the Tris-HCl (15 mM) buffered experimental medium. The medium (*p*H 7.2, 320 mOsm) also contained (mM): NaCl, 137; KCl, 5.3; $MgCl_2$, 0.8; and glucose, 5.6. DRG neurone somatic action potentials are dependent on both sodium and calcium[25–27,30]. TEA, which decreases voltage-dependent potassium conductance[31], enhanced the calcium component of DRG neurone action potentials. In TEA-containing medium, DRG neurone action potentials have a sodium component occupying approximately the first millisecond with the remaining broad plateau dependent on calcium[26]. Preliminary investigation[32] was performed using superfusion of medium containing 18 mM TEA. The lower TEA concentrations used in the presently described experiments increased the viability of DRG neurones and increased the stability of action potential duration.

For experiments, aliquots of morphiceptin (Peninsula) and Leu-enkephalin (Calbiochem-Behring) dissolved in distilled water at 1 mM and frozen in plastic tubes were thawed and serially diluted in experimental medium to appropriate concentrations. Morphiceptin and Leu-enkephalin were applied to single neurones by pressure ejection (0.5–2.0 p.s.i.) from micropipettes with tip diameters of 2–5 μm positioned ~5 μm from the DRG neurone. Opioid peptide application was for 1 s and was delivered 3–4 s before evoking an action potential. In these

0028–0836/82/420730—04$01.00

conditions, pressure ejection of opioid peptide-free medium did not decrease the calcium component of action potentials. Additional micropipettes (up to three) with tip diameters of 15–25 μm were used to apply naloxone (Endo) by diffusion when positioned 10–15 μm from DRG neurones. The procedure followed was first to obtain a control response following application of opioid peptide. A naloxone-containing micropipette was then positioned near the DRG neurone, and the antagonist allowed to diffuse into the medium surrounding the cell for 1 min before reapplication of opioid peptide. Finally, the naloxone containing micropipette was removed from its position near the DRG neurone, and the opioid peptide was re-applied. Thus, single DRGs could be characterized in terms of sensitivity to morphiceptin and Leu-enkephalin as well as naloxone concentrations required for antagonism.

In initial studies with morphiceptin, we determined that the opioid peptide decreased DRG neurone somatic calcium-dependent action potential duration dose dependently and over a concentration range similar to that of Leu-enkephalin (100 nM–10 μm)[10]. As with Leu-enkephalin, DRG neurone sensitivity to morphiceptin varied considerably across cells with shortening of calcium-dependent action potentials by 1 μm ranging over 2–65%. Comparable maximum decreases in action potential duration were obtained for 1 μM Leu-enkephalin and morphiceptin.

We then compared the actions of morphiceptin and Leu-enkephalin at equimolar concentrations (over 500 nM–5 μM) on somatic calcium-dependent action potentials in single DRG neurones ($n = 133$ of which 98 responded). Opioid peptide effects on action potentials ranged from Leu-enkephalin producing large decreases and morphiceptin not affecting the calcium component of action potentials (Fig. 1*a*i) to equal reduction of action potential duration by both opioid peptides (Fig. 1*a*ii). Of the 98 DRG neurones that responded to morphiceptin or Leu-enkephalin, 42 had decreases in action potential duration of at least 20%. Of these 42 neurones, the distribution of relative DRG neurone response to morphiceptin and Leu-enkephalin was as follows (Fig. 1*b*): four DRG neurones responded only to Leu-enkephalin but not to morphiceptin, 30 responded to both peptides but better to Leu-enkephalin than to morphiceptin, five responded equally well to both peptides, and only three DRG neurones responded slightly better to morphiceptin than to Leu-enkephalin (Fig. 1*b*). The distribution of relative response of individual DRG neurones to morphiceptin and Leu-enkephalin was not altered by inclusion of the 56 neurones which showed small responses to the opioid peptides.

DRG neurones characterized by differential sensitivity to morphiceptin and Leu-enkephalin were additionally tested for sensitivity of the opioid peptide actions to naloxone antagonism. Naloxone antagonized decreases in calcium-dependent action potential duration dose dependently with reliable blockade of 500 nM to 5 μM morphiceptin responses produced by naloxone concentrations as low as 5–10 nM (Fig. 2*a*i; *b*, closed triangles). Responses to 500 nM to 5 μM morphiceptin were partially attenuated by naloxone application at a concentration 1/1,000th the morphiceptin concentrations ($n = 5$), almost entirely abolished by naloxone concentrations 1/100th of the morphiceptin concentration ($n = 10$), and completely reversed by naloxone at 1/10th of the morphiceptin concentration ($n = 13$) (Fig. 2*b*, closed circles). However, naloxone sensitivity of Leu-enkephalin mediated decreases in somatic calcium-dependent action potential duration was variable with responses to 200 mM–1 μM Leu-enkephalin reversed by 5–10 nM naloxone in some DRG neurones (Fig. 2*a*i; *b*, closed circles) but requiring 1 μM naloxone for antagonism in other DRG neurones (Fig. 2*a*ii; *b*, open circles). The sensitivity of Leu-enkephalin responses to naloxone antagonism correlated with DRG neurone responsiveness to morphiceptin. Leu-enkephalin responses were reversed by low concentrations of naloxone in DRG neurones which responded well to morphiceptin (Fig. 2*a*i; *b*, closed circles), with antagonism occurring at a ratio of naloxone concentration to opioid peptide concentration of 1 : 10 ($n = 2$) and 1 : 100 ($n = 3$) (Fig. 2*b*). In contrast, high concentrations of naloxone were required for antagonism of Leu-enkephalin responses in DRG neurones which responded poorly to morphiceptin (Fig. 2*a*ii; *b*, open circles). Naloxone only slightly attenuated Leu-enkephalin responses when applied at a concentration 1/100th ($n = 6$) or 1/10th ($n = 6$) the Leu-enkephalin concentration, moderately attenuated responses when applied at a concentration equal to the peptide concentration ($n = 10$), and totally abolished Leu-enkephalin responses when the naloxone concentration applied exceeded the opioid peptide concentration ($n = 8$) (Fig. 2*b*, open circles).

We observed a subpopulation of DRG neurones which responded well to opioid peptides which may correlate with highly specific opiate effects on sensory transmission. In neurones which responded, the pattern of response to morphiceptin and Leu-enkephalin was heterogeneous. Morphiceptin and Leu-enkephalin decreased the duration and amplitude of somatic calcium-dependent action potentials equipotently in a proportion of DRG neurones, consistent with mediation by μ-receptors. Two additional findings support mediation by μ-receptors. First, large decreases in DRG neurone somatic calcium-dependent action potential duration were produced by 1 μM morphiceptin, a concentration more than 1 log unit below the half-maximal effective dose in the δ-receptor-predominated mouse vas deferens[11]. Second, DRG neurones which responded equipotently to morphiceptin and Leu-enkephalin were highly sensitive to naloxone antagonism. In contrast, a proportion of

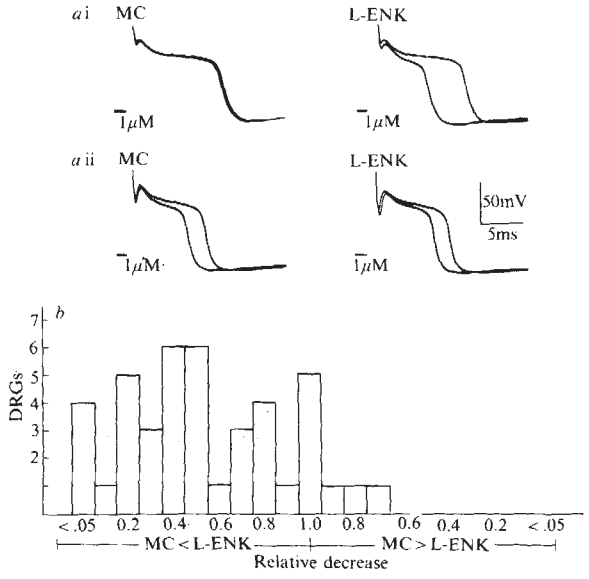

Fig. 1 Heterogeneous response pattern of DRG neurones to morphiceptin and Leu-enkephalin. *a*, *a*i and *a*ii illustrate heterogeneous response pattern to morphiceptin (MC) and Leu-enkephalin (L-ENK) observed on DRG neurone somatic calcium-dependent action potentials. Action potentials were evoked from resting membrane potential at 15-s intervals by depolarizing current pulses. In this and the following figure, data shown are superimposed action potentials evoked before (longer duration action potential) and after (shorter duration action potential) opioid peptide application. In all cases action potential duration gradually returned to baseline over 1–2 min. Data shown in *a*i and *a*ii are successive recordings from two DRG neurones in the same culture using the same opioid peptide-containing micropipettes. Care was taken to position the morphiceptin- and Leu-enkephalin-containing micropipettes at similar positions near DRG neurones. *b*, Summary of data comparing potency of morphiceptin and Leu-enkephalin on 42 DRG neurones each of which responded with a decrease in action potential duration of at least 20% to Leu-enkephalin or morphiceptin. Data are plotted as relative decrease in action potential duration produced by morphiceptin and Leu-enkephalin on single DRG neurones. A value of 1.0 indicates that morphiceptin and Leu-enkephalin decreased action potential duration equipotently ($n = 5$). To the left of 1.0, morphiceptin was less potent than Leu-enkephalin in decreasing action potential duration with the fraction being DRG neurone response to morphiceptin divided by the response to Leu-enkephalin. To the right of 1.0, morphiceptin was more potent than Leu-enkephalin, and the fractions represent DRG neurone response to Leu-enkephalin divided by the response to morphiceptin.

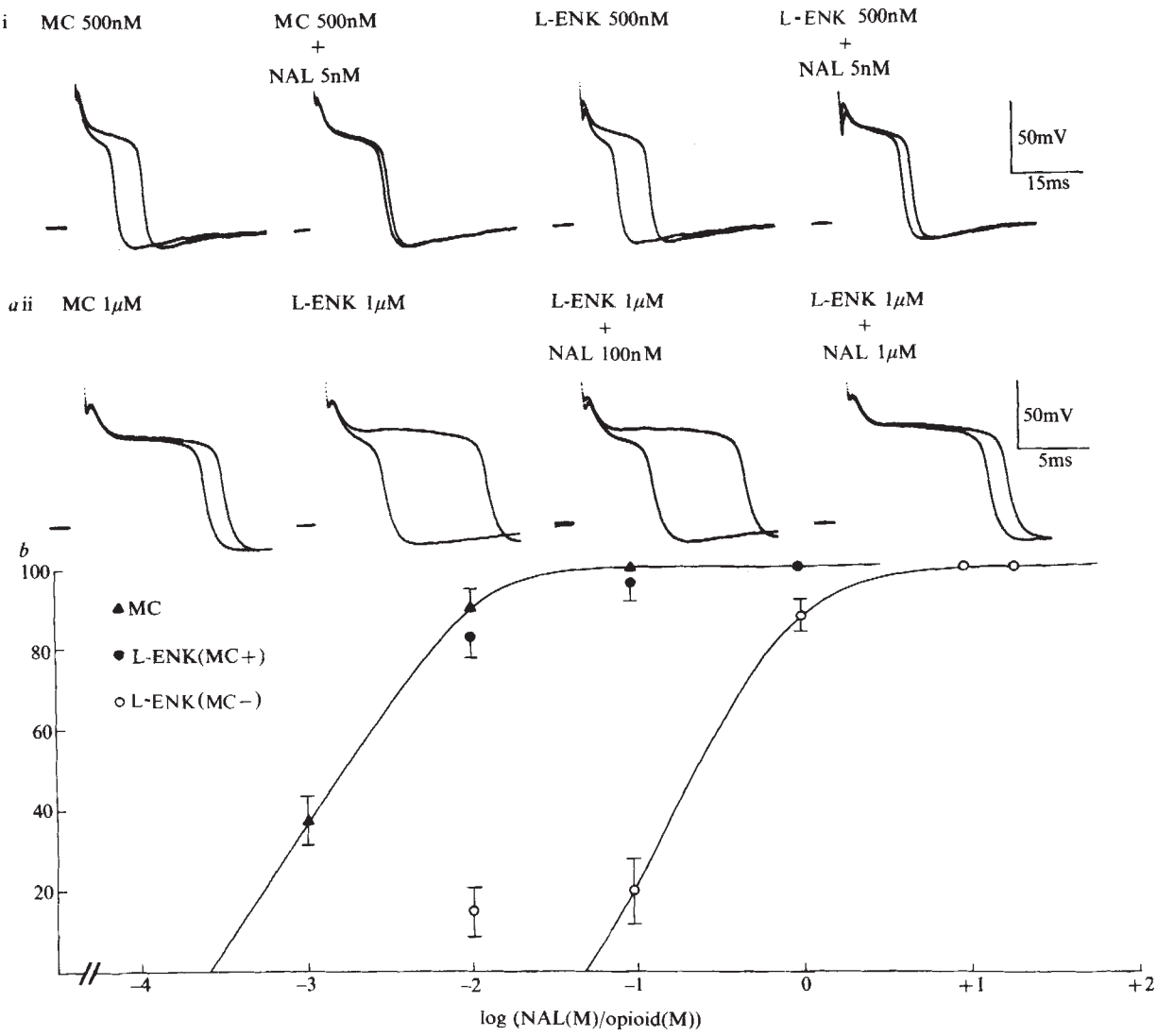

Fig. 2 Morphiceptin-induced decreases in calcium-dependent action potential duration were highly sensitive to naloxone antagonism while higher naloxone concentrations were required to reverse Leu-enkephalin produced decreases of action potential duration in DRG neurones which did not respond to morphiceptin. Single DRG neurones were characterized in terms of sensitivity to Leu-enkephalin and morphiceptin and then the sensitivity of the opioid peptide responses to antagonism by naloxone was tested. *a*, *ai* and *aii* illustrate DRG neurones with a different response pattern to Leu-enkephalin and morphiceptin and with opioid peptide responses which had differential naloxone sensitivity. *ai*, A DRG neurone which responded approximately equally well to morphiceptin and Leu-enkephalin. Both morphiceptin and Leu-enkephalin responses were highly sensitive to naloxone antagonism. *aii*, A DRG neurone which responded well only to Leu-enkephalin but not morphiceptin. Antagonism of Leu-enkephalin response required higher naloxone concentration. *b*, Summary of sensitivity to naloxone antagonism of Leu-enkephalin and morphiceptin responses. The ordinate is the per cent decrease in response to the opioid peptides produced by naloxone (NAL). The abscissa is the log of the ratio of naloxone to opioid peptide concentration. For example, a log (NAL/OPIOID) of zero indicates equimolar concentrations of naloxone and opioid peptides, a negative one indicates naloxone at 1/10th the opioid peptide concentration, and a positive one indicates naloxone at 10 times the opioid peptide concentration. Morphiceptin and Leu-enkephalin concentrations ranged over 200 nM–5 μM. ▲, Morphiceptin responses, highly sensitive to naloxone antagonism with almost 100% antagonism at a naloxone to morphiceptin concentration ratio of 0.01. Sensitivity of Leu-enkephalin responses to antagonism by naloxone was correlated with DRG neurone responsiveness to morphiceptin. ●, Leu-enkephalin responses in DRG neurones in which the ratio of response to morphiceptin to response to Leu-enkephalin was at least 0.75 (MC^+) were approximately as sensitive as morphiceptin-responses to naloxone antagonism. In contrast, Leu-enkephalin responses (○) in DRG neurones in which the ratio of response to morphiceptin to response to Leu-enkephalin was less than 0.25 (MC^-) were about 100-fold less sensitive to naloxone antagonism with 100% antagonism not occurring until naloxone concentrations exceeded Leu-enkephalin concentrations. Bars are standard error of the mean.

DRG neurones were highly sensitive to Leu-enkephalin, did not respond to morphiceptin and required high concentrations of naloxone for antagonism of Leu-enkephalin responses, consistent with the opiate effects being mediated by δ-receptors.

Therefore, our results suggest that both μ- and δ-receptors mediate decreases in somatic calcium-dependent action potentials of DRG neurones. If opiate interaction with these receptors at primary afferents produces a similar effect on calcium entry, the result would be decreased transmitter release. Thus, both μ- and δ-receptors would act to depress transmitter release from primary afferent terminals just as both receptor types have inhibitory actions on myenteric neurones[15]. Finally, the heterogeneous pattern of response to morphiceptin and Leu-enkephalin support a variable proportion of μ- and δ-receptors on DRG neurones, suggestive of distinct roles for these receptors in the processing of information at the level of the spinal cord.

We thank Patricia Szczepanski-Marquardt for her maintenance of the neuronal cell cultures and Kathy Lundquist for her secretarial assistance. M.A.W. was supported by NIMH-NRSA 12479 Fellowship and by NIDA-DA5244. R.L.M. was supported in part by NIH Research Career Development Award NS 00480. The research was supported in part by NIH grant NS 15225 and NIDA-DA5244.

Received 23 March; accepted 2 August 1982.

1. Fields, H. L., Emson, P. C., Leigh, B. K., Gilbert, R. F. T. & Iversen, L. L. *Nature* **284**, 351–353 (1980).
2 Gamse, R., Holzer, P. & Lembeck, F. *Naunyn-Schmiedeberg's Archs Pharmak.* **308**, 281–285 (1979).
3. Lamotte, C., Pert, C. B. & Snyder, S. H. *Brain Res.* **112**, 407–412 (1976).
4. Calvillo, O., Henry, J. L. & Neuman, R. S. *Can. J. Physiol. Pharmac.* **52**, 1207–1211 (1974).

5. Duggan, A. W., Hall, J. G. & Headley, P. M. *Nature* **264**, 456–458 (1976).
6. Duggan, A. W., Hall, J. G. & Headley, P. M. *Br. J. Pharmac.* **61**, 65–76 (1977).
7. Macdonald, R. L. & Nelson, P. G. *Science* **199**, 1449–1451 (1978).
8. Mudge, A. W., Leeman, S. E. & Fischbach, G. D. *Proc. natn. Acad. Sci. U.S.A.* **76**, 526–530 (1979).
9. Sastry, B. R. *Neuropharmacology* **18**, 367–375 (1979).
10. Werz, M. A. & Macdonald, R. L. *Brain Res.* **239**, 315–321 (1982).
11. Chang, K.-J., Killian, A., Hazum, E., Cuatrecasas, P. & Chang, J.-K. *Science* **212**, 75–77 (1981).
12. Chang, K.-J. & Cuatracasas, P. *J. biol. Chem.* **254**, 2610–2618 (1979).
13. Chang, K.-J., Cooper, B. R., Hazum, E. & Cuatrecasas, P. *Molec. Pharmac.* **16**, 91–104 (1979).
14. Chavkin, C. & Goldstein, A. *Nature* **291**, 591–593 (1981).
15. Egan, T. M. & North, R. A. *Science* **214**, 923–924 (1981).
16. Goodman, R. R., Snyder, S. H., Kuhar, M. J. & Young, W. S. III *Proc. natn. Acad. Sci. U.S.A.* **77**, 6239–6243 (1980).
17. Kosterlitz, H. W., Lord, J. A. H., Paterson, S. J. & Waterfield, A. A. *Br. J. Pharmac.* **68**, 333–342 (1980).
18. Lord, J. A. H., Waterfield, A. A., Hughes, J. & Kosterlitz, H. W. *Nature* **267**, 495–499 (1977).
19. Martin, W. R. *Pharmac. Rev.* **19**, 463–521 (1967).
20. Robson, L. E. & Kosterlitz, H. W. *Proc. R. Soc.* B**205**, 425–432 (1979).
21. Schulz, R., Wüster, M., Krenss, H., Herz, A. *Molec. Pharmac.* **18**, 395–401 (1980).
22. Waterfield, A., Smokcum, R. W. J., Hughes, J., Kosterlitz, H. W. & Henderson, G. *Eur. J. Pharmac.* **43**, 107–116 (1977).
23. Llinas, R., Steinberg, I. Z. & Walton, K. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2918–2922 (1976).
24. Llinas, R., Steinberg, I. Z. & Walton, K. *Biophys. J.* **33**, 323–352 (1981).
25. Dichter, M. A. & Fischbach, G. D. *J. Physiol., Lond.* **267**, 281–298 (1977).
26. Heyer, E. J. & Macdonald, R. L. *J. Neurophysiol.* **47**, 641–655 (1982).
27. Ransom, B. R. & Holz, R. W. *Brain Res.* **136**, 445–453 (1977).
28. Ransom, B. R., Neale, E., Henkart, M., Bullock, P. N. & Nelson, P. G. *J. Neurophysiol.* **40**, 1132–1150 (1977).
29. Zhang, A.-Z., Chang, J.-K. & Pasternak, G. W. *Life Sci.* **28**, 2829–2836 (1981).
30. Yoshida, S. & Matsuda, Y. *J. Neurophysiol.* **42**, 1134–1145 (1979).
31. Armstrong, C. M. & Binstock, L. *J. gen. Physiol.* **48**, 859–872 (1965).
32. Werz, M. A. & Macdonald, R. L. *Neurosci. Abstr.* **6**, 416 (1980).

GABA directly affects electrophysiological properties of pituitary pars intermedia cells

P. S. Taraskevich & W. W. Douglas

Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510, USA

Evidence that the endocrine cells of the pars intermedia of the mammalian pituitary gland secrete not only melanotropins but corticotropins and endorphins[1] heightens interest in the nervous control of these cells. Within the mammalian adenohypophysis the pars intermedia is unique in being directly innervated by neurones whose cell bodies lie in the brain[2]. However, the nature and function of this innervation is poorly understood. A dopaminergic tract has been identified in rats[3,4], which appears to have an inhibitory function[5], and dopamine applied directly to isolated rat pars intermedia cells inhibits both the discharge of spontaneous action potentials[6] and secretion[7,8]. In addition, recent immunohistochemical studies in rats indicate that central neurones which apparently contain γ-aminobutyric acid (GABA) also project to the pars intermedia[9,10]. Here we report that in the same species GABA directly affects the electrophysiological properties of endocrine cells isolated from the pars intermedia and that the ionic and pharmacological characteristics of this action of GABA resemble those encountered at many GABAergic synapses in the central nervous system (CNS). We conclude that the brain can influence the endocrine cells of the pars intermedia directly through GABAergic mechanisms.

Isolated endocrine cells of rat pars intermedia maintained *in vitro* spontaneously discharge action potentials[6] which are Na spikes with a Ca component[11]. In such cells, GABA (2×10^{-6} or 1×10^{-5} M) elicited a brief flurry of action potentials of diminishing amplitude, followed by arrest of action potentials for as long as exposure to GABA was maintained (up to 60 s). Shortly after the removal of GABA, action potentials reappeared and increased in amplitude to reach the pre-GABA levels (Fig. 1*a*). Further analysis (Fig. 1*b*) showed that GABA rapidly and reversibly depolarized the cells and profoundly reduced membrane resistance—to $11\pm2.1\%$ (mean ± s.e.m., $n = 31$) of the control values with 10^{-4} M GABA. Such changes in membrane potential and resistance explain the ability of GABA to stimulate and arrest spike discharge and reduce spike amplitude.

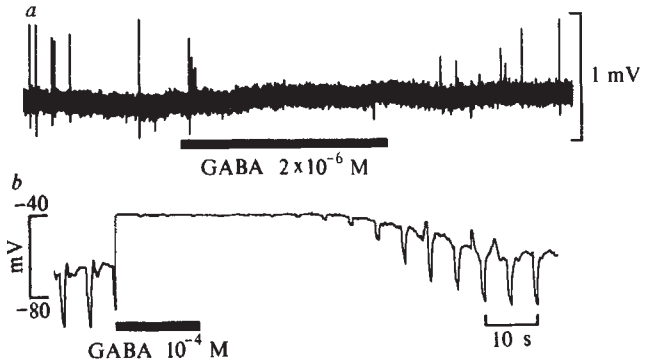

Fig. 1 Typical effects of GABA on electrophysiological properties of isolated pars intermedia cells from rats. *a*, Effect of GABA on spontaneous action potential activity recorded extracellularly. GABA, applied to the cell for the time indicated by the bar, initially caused a brief burst of action potentials followed by cessation of action potential activity. Note that the amplitude of the action potentials wanes during the initial burst and waxes during the recovery from exposure to GABA. *b*, Effect of GABA on membrane potential and resistance recorded intracellularly. In this record, and in those shown in Fig. 2, membrane resistance is assessed at regular intervals (~5 s) by passing brief inward (hyperpolarizing) current pulses of constant magnitude. The amplitude of the resulting hyperpolarizing responses thus reflects membrane resistance such that a reduction in amplitude signals a fall in resistance. GABA produced an immediate depolarization accompanied by a profound reduction in membrane resistance which lasted, in this cell, for many seconds after GABA was withdrawn. In other cells recovery from GABA was more rapid (compare with Fig. 2*d*). The cells used in this and all other experiments were endocrine cells dispersed from the pars intermedia of rat neurointermediate lobes, maintained in short-term culture (<14 days) and recorded from as previously described[6]. The solution in which the cells were bathed during recording contained (mM): NaCl, 150; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1; glucose, 11; HEPES, 5 (*p*H 7.1). GABA, and all other drugs, were delivered in this solution to the cells by a gravity flow pipette (inner tip diameter about 10 μm) which effectively perifuses the cell.

The collapse of membrane resistance and potential in the pars intermedia cells in response to GABA seems to result from an increased Cl^- conductance. This is indicated by the dependence of the null potential of the GABA response on the extracellular concentration of Cl^- ions (Fig. 2*a*,*b*). Such an effect, to increase Cl^- conductance, is now a familiar action of GABA at sites of GABAergic transmission within the brain and spinal cord[12–14]. At many such sites, the Cl^--dependent responses to GABA are inhibited by bicuculline and potentiated by diazepam[12–16]. We found that these drugs similarly affected the responses of the pars intermedia cells to GABA (Fig. 2*c*–*f*). Bicuculline, given with GABA in equimolar amount (10^{-4} M), potently, rapidly and reversibly antagonized the GABA response, both the fall in membrane resistance and membrane potential being greatly abbreviated or abolished (Fig. 2*c*,*d*). Even when given in a 10-fold lower concentration (10^{-5} M), bicuculline markedly inhibited the response to GABA (10^{-4} M), although a vestige of the response was then regularly apparent in an early, short-lived (5–15 s) phase of reduced membrane resistance and potential. Diazepam (2×10^{-5} M), by contrast, rapidly and reversibly potentiated responses to GABA. This effect was prominent (Fig. 2*e*,*f*) when GABA was used in clearly submaximal concentrations: responses to maximally effective concentrations of GABA were little, if at all, affected by diazepam. Neither bicuculline nor diazepam by itself had any apparent effect on resting membrane potential or resistance.

In functioning as the principal inhibitory transmitter at central synapses, GABA often acts to increase conductance to Cl^- ions. This action can produce two prominent effects[12–14]. First, acting directly on the nerve cell body, GABA can reduce excitability and spontaneous activity. Clearly, there is a parallel here in the behaviour of the pars intermedia cells (Fig. 1*a*). Second, acting on presynaptic terminals, GABA can diminish the depolarizing stimulus to secretion (the action potential) and thereby reduce transmitter output. Here, again, we obtained

0028–0836/82/420733—02$01.00