Br. J. Pharmac. (1986), **88**, 87–93

Inhibitory actions of opioid compounds on calcium fluxes and neurotransmitter release from mammalian cerebral cortical slices

H.F. Bradford[1], J.M. Crowder & E.J. White

Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ

1 The effects of opioid agonists on veratrine-stimulated Ca^{2+} influx and amino acid neurotransmitter release in rat cerebrocortical brain slices were studied. Inhibitory effects were seen on both of these parameters with all of the opioid agonists used.

2 None of the drugs used affected basal $^{45}Ca^{2+}$ uptake, basal K^+ content or basal amino acid release from the slices.

3 At high concentrations (100 μM) fentanyl, tifluadom, U50,488H, butorphanol and bremazocine greatly inhibited the depolarization of the slices by veratrine as determined by the reduced release of K^+.

4 The opioid receptor subtypes at which the drugs were acting were characterized by the antagonistic effects of naloxone and WIN44441-3.

5 The opioid-induced inhibition of stimulated Ca^{2+} uptake and amino acid release were not antagonized by WIN44441-2, the inactive enantiomer of WIN44441-3.

6 It is concluded that opioid agonists acting through μ- and κ-receptors and probably through δ- and σ-receptors, have an inhibitory effect on Ca^{2+} uptake into cerebrocortical brain slices and the subsequent release of aspartate, glutamate and γ-aminobutyric acid (GABA).

Introduction

Previously, in our laboratory, morphine has been shown to inhibit depolarization-induced calcium influx and neurotransmitter release from rat cortical synaptosomes (Dhaliwal & Bradford, 1982). This effect was stereospecific and reversed by relatively low concentrations of naloxone, therefore morphine is likely to be acting through the μ-type opioid receptor. The inhibitory action of morphine on calcium influx and neurotransmitter release was postulated to be directed at the calcium channel.

As an extension of this work, a number of opioid agonists and antagonists have been studied to determine whether occupation of any other opioid receptor subtypes might effect calcium influx and amino acid neurotransmitter release from slices of cerebral cortex, either at rest or during periods of stimulation with veratrine.

[1]Author for correspondence

Methods

Preparation and incubation of brain slices

Brain slices (approx. 1.0 × 0.5 cm and 0.3 mm thick) were prepared from rat cerebral cortex using a McIlwain recessed guide and a razor blade (McIlwain, 1961). These were incubated at 37°C for 30 min in 5 ml glass beakers each carrying a gassing line which delivered pure O_2. The relevant opioid compound was added to the incubation medium (composition, mM: NaCl 138, KCl 5, $MgSO_4$ 1.0, $CaCl_2$ 1.2, NaH_2PO_4 1.2, Tris 20, glucose 10, pH 7.4), dissolved in 200 μl of this medium. When the effect of an opioid antagonist was studied, it was included in the incubation medium at the required concentration before the addition of the slice.

Uptake of $^{45}Ca^{2+}$

Five minutes after the opioid compound was added,

$^{45}CaCl_2$ (Amersham International) was added (200 μl) to each beaker to give a final specific activity of 0.14 Ci mol^{-1}. Veratrine HCl (200 μl) was added 30 s later to give a final concentration of 25 μM. The final volume of the incubation medium was 4.5 ml. After a total period of incubation with $^{45}Ca^{2+}$ of 12 min, the slices were removed and each was immersed in 2 ml EGTA stopping solution (composition, mM: NaCl 120, KCl 5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, Tris 20, EGTA 30, pH 7.4) at room temperature. The slices were each transferred in quick succession through 3 × 2 ml K^+ free washing medium (composition, mM: NaCl 137,-$MgCl_2$ 1.2, NaH_2PO_4 1.2, Tris 20, glucose 10, pH 7.4). They were then made soluble in 1 ml of 1.0 M NaOH for 30 min at 50°C.

Measurement of $^{45}Ca^{2+}$

An aliquot of dissolved tissue was added to a liquid scintillation mixture (Packard 199) and counted in a Packard 300CD Tricarb liquid scintillation counter to determine the tissue content of radioactive calcium.

Measurement of K^+

K^+ was measured in the dissolved slices by atomic absorption spectrophotometry, using a SB-900 Atomic Absorption Spectrophotometer (G.B.C. Scientific Equipment, Victoria, Australia). Standard solutions of K^+ in 1 M NaOH were used to quantitate the K^+ levels.

Measurement of amino acids

At the end of the incubation, aliquots of the incubation medium were acidified with 0.025 M HCl containing the appropriate concentration of norleucine as internal standard. Amino acids were measured with a chromaspek J180 with fluorimeter J143 (Rank-Hilger, Margate, Kent). An acid buffer (0.05 M citric acid, 0.15 M LiCl) was titrated against a basic buffer (0.05 M boric acid, 0.2 M LiOH) using electronic programmed gradient elution from pH 2 to pH 12. The run time was 2 h. Orthophthaldehyde with mercaptoethanol (800 mg and 2 ml respectively in 1 litre distilled water) was used as the fluorogenic agent for analysis of the amino acids. This was carried out in 0.3 M $NaHCO_3$ buffer, pH 10. The column was 15 cm × 3 mm packed with 4 μ of 8% cross-linked polystyrene sulphonic acid resin.

Measurement of proteins

Protein levels were measured using the method of Lowry *et al.* (1951), Bovine serum albumin was used for the standards.

Opioid drugs

We should like to thank the following companies for their kind gifts: etorphine HCl (C-Vet Ltd); butorphanol tartrate (Bristol-Myers Co.); fentanyl citrate, sufentanil citrate (Janssen Pharmaceutica); U-50,488H (Upjohn Co.); bremazocine HCl, tifluadom HCl (Sandoz Ltd); ethylketocyclazocine methane sulphonate, pentazocine HCl, WIN44441-3, WIN44441-2 (Sterling-Winthrop); SKF 10,047 (NIDA); nalbuphine HCl, naloxone HCl (Du Pont).

Results

The effects of opioid agonists on Ca^{2+} uptake and depolarization

All of the drugs employed inhibited veratrine-stimulated $^{45}Ca^{2+}$ uptake, though with varying potencies (Table 1). (Neither basal $^{45}Ca^{2+}$ uptake nor basal K^+ content of brain slices were altered by any of these drugs).

At higher concentrations (e.g. 100 μM) fentanyl, tifluadom, U50,488H (trans-(±)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl] benzeneacetamide methane sulphonate), butorphanol and bremazocine greatly reduced the veratrine-induced release of K^+ from the slices as well as inhibiting $^{45}Ca^{2+}$ uptake, and could therefore be exerting at least part of their actions at these higher concentrations by reducing the depolarizing action of veratrine (Table 1).

The opioid agonists are classified in Table 1 according to the receptor for which they appear to have greatest selectivity in binding studies (James & Goldstein, 1984). However, considerable cross-reactivity between the receptor types occurs. Therefore etorphine, for example, may cross-react with δ- and κ-receptors to some extent (Rosenbaum *et al.*, 1984). Opioid antagonists were employed to specify further the opioid receptor types which are involved in mediating these effects on calcium influx.

Naloxone and WIN44441-3 antagonism of the effects on $^{45}Ca^{2+}$

Naloxone is primarily a μ-antagonist, but it is also capable of antagonizing κ- and δ-agonist activity, the order of potency being $\mu > \kappa > \delta$ (Takemori & Portughese, 1984). WIN44441-3 [(2d, 6d, 115*)-(−)-1 cyclopentyl-5-(1, 2, 3, 4, 5, 6-hexahydro-8-hydroxy-3, 6, 11-trimethyl-2, 6-methano-3-benzazocin-11-yl)-3 pentanone methane sulphonate] is a pure opioid antagonist which is thought to act with a greater selectivity for the κ-receptor (Romer *et al.*, 1980) but also acts on μ- and δ-receptors (Ward *et al.*, 1983).

All the μ-agonists in this study were effectively

Table 1 The effects of opioid drugs on calcium uptake into cerebrocortical slices and depolarization stimulated by veratrine

Opioid agonists (μM)		*Inhibition of Ca^{2+} uptake* (%)*	*Inhibition of K^+ loss† (%)*	*% opioid antagonism‡ Naloxone* (μM) *1*	*10*	*100*	*WIN44441-3* (μM) *1*	*10*	*100*
μ-agonists									
Morphine	100	122	0	94					49
	10	85	0	114					22
	1	14	0	100					0
Etorphine	100	114	0	77					38
	41	93	17	98					47
	10	75	0	106					30
	1	32							
	0.1	0.2							
Fentanyl	100	102	113	1					10
	10	115	50	0				1	
	1	57	0	12	41			0	0
Sufentanil	100	111	0	28				0	
	1	96	0	93	102			3	25
κ-agonists									
Tifluadom	100	96	77		0			0	
	10	94	16		0			18	
	1	78	10		0	0		77	
	0.1	36	2			0		82	
U50,488H	100	87	89		22		76		
	10	68	8		3		91		
δ-agonist									
[D-Ala^2,Met^5]	100	80	0	0				0	
enkephalinamide	20	0	6	0				0	
σ-agonist									
SKF 10,047	100	106	0	0	7			2	
	10	52	32	0					
κ-agonist/μ-antagonists									
Ethylketo-cyclazocine	100	100	0		8				3
	41	103							
	12	93							
	10	78	0		25		34	105	
	3	56					100		
	1	31	0		144		123	97	
	0.3	4							
Pentazocine	100	61	0			8		40	
	10	56	0			11		78	
	1	30	0			57		100	
Nalbuphine	100	95	3			0		0	
	10	76	0			0		27	
Bremazocine	100	100	64					87	
	10	84	0		20		50	91	
	1	69	0			96	83		
Butorphanol	100	103	55						
	41	116	13		65		97		
	12	82	0		53		129		
	4	25							
	1	10						47	

Results are mean of 6 incubations from 2 experiments; s.d. values are all within 10% for Ca^{2+} content.
*Basal Ca^{2+} uptake 1424 ± 106 n mol 100 mg^{-1} protein 12 min^{-1}; 25 μM veratrine stimulated Ca^{2+} uptake 2278 ± 202 n mol 100 mg^{-1} protein 12 min^{-1}.
†Basal K^+ content 89.7 ± 0.3 μ equivalent 100 mg^{-1} protein; 25 μM veratrine stimulated K^+ content 40.1 ± 0.7 μ equivalent 100 mg^{-1} protein.
‡% opioid antagonism of effects on stimulated Ca^{2+} uptake.

antagonized by 1 μM naloxone except fentanyl (Table 1). However fentanyl, like the other μ-agonists was not effectively antagonized by 100 μM WIN44441-3. These results suggest that the actions of morphine, etorphine and sufentanil are primarily at the μ-receptor rather than the κ-receptor. The actions of fentanyl may also be through the μ-receptor, but are unlikely to be through the κ-receptor.

Tifluadom and U50,488H were both preferentially antagonized by WIN44441-3 rather than naloxone, indicating that they act primarily at the κ-receptor in our assay system. Both these compounds have been found to be very selective κ-agonists in other systems (Vonvoigtlander *et al.*, 1982; Romer *et al.*, 1982).

Table 2 The effects of opioid drugs on glutamate release from cerebrocortical slices stimulated by veratrine

Opioid agonists (μM)		*% inhibition of stimulated glutamate release**	*% opioid antagonism‡* *Naloxone* (μM) *1*	*10*	*100*	*WIN44441-3* (μM) *1*	*10*	*100*
μ-agonists								
Morphine	100	150	117					22
	10	116	118					89
	1	36	100					23
Etorphine	100	82	72					0
	10	62	96					90
	1	28	152					83
Fentanyl	100	ND	ND					ND
	10	ND	ND				ND	
	1	108	0	66			0	1
Sufentanil	100	ND	ND					
	1	157	138	84			0	39
κ-agonists								
Tifluadom	100	98		0			0	
	10	86		0			22	
	1	54		0	2		96	
	0.1	41			0		117	
U50,488H	100	98		0		77		
	10	70		0		131		
δ-agonist								
[D-Ala^2,Met^5] enkephalinamide	100	45	6				0	
	20	ND	ND				ND	
σ-agonist								
SKF 10,047	100	108	ND	ND			ND	
	10	102	19					
κ-agonist/μ-antagonists								
Ethylketo-cyclazocine	100	85		4				25
	10	71		85			99	
	1	43		99		100	100	
Pentazocine	100	98			0		32	
	10	66			26		67	
	1	30			53		100	
Nalbuphine	100	100			8		42	
	10	92			36		87	
Bremazocine	100	101					93	
	10	48		66		97	113	
	1	30			73	116		
Butorphanol	100	103						
	41	109		48		100		
	12	100		67		122		
	4	86				125		

Results are mean of 6 incubations from 2 experiments; s.d. values are all within 10% of the mean for glutamate release.
*Basal glutamate release 1806 ± 184 n mol 100 mg^{-1} protein; 25 μM veratrine-stimulated glutamate release 2972 ± 165 n mol 100 mg^{-1} protein.
‡% opioid antagonism of effects on stimulated glutamate release.
ND not determined.

Table 3 Comparison of the effect of WIN44441 isomers on the actions of 4 opioid agonists

Opioid agonist	*% inhibition of veratrine stimulated Ca^{2+} uptake*	*% opioid antagonism WIN44441-2 (+)*	*WIN44441-3 (−)*	*% inhibition of veratrine-stimulated glutamate release*	*% opioid antagonism WIN44441-2 (+)*	*WIN44441-3 (−)*
Morphine	112	0	49	150	0	22
Etorphine	114	0	38	82	0	0
Ethylketocyclazocine	100	4	31	85	0	25
Pentazocine	61	2*	40*	98	0*	32*

*To obtain these values 10 μM WIN44441-2 and WIN44441-3 was used; 100 μM of these isomers was used for the other values. The stimulating agent was 25 μM veratrine.

[D-Ala^2, Met^5] enkephalinamide and SKF 10,047 were not significantly antagonized by naloxone (1 μM) or WIN44441-3 (10 μM). This suggests that these agonists may be acting at opioid receptor types other than μ and κ. Since δ- and σ-selective antagonists (Cotton *et al.*, 1984; Tam, 1985) were not employed, the actions of these drugs through δ- and σ-receptors respectively could not be confirmed.

Sufentanil is thought to be very selective for μ-receptors over κ- and δ-receptors (Goldstein & James, 1984), but 100 μM WIN44441-3 partially antagonized the inhibition of Ca^{2+} influx by 1 μM sufentanil. This suggests that WIN44441-3 may not be absolutely specific for the κ-receptor, but certainly has a greater antagonistic activity at κ- over μ-receptors in these experimental conditions.

The effects of opioid agonists and antagonists on amino acid release

As previously found, veratrine caused differential tetrodotoxin-sensitive release of glutamate, aspartate and GABA from nerve terminals (Wedege *et al.*, 1977). There was no effect on amino acids such as threonine, which does not have the properties of a neurotransmitter. Basal neuroactive amino acid release was unaffected by the drugs used. However, opioid agonists did inhibit release stimulated by veratrine (25 μM) to various degrees. These actions are expressed in terms of their influence on glutamate release (Table 2), though very similar suppression of veratrine evoked-aspartate and γ-aminobutyric acid (GABA) release also occurred.

Actions of isomers of WIN44441

Table 3 shows the lack of effect that WIN44441-2 (the (+) optical isomer of WIN44441-3) had on the inhibition of stimulated Ca^{2+} uptake and neurotransmitter release by 4 opioid agonists. WIN44441-2 had no action on veratrine-stimulated depolarization or on the basal levels of Ca^{2+} uptake, K^+ content or amino acid release.

Discussion

Opioid receptor subtypes have been identified by autoradiography in rat brain cortex, using selective ligands (Chang *et al.*, 1979; Quirion *et al.*, 1981; Goodman & Snyder, 1982).

In our study all the different receptor agonists examined appeared to affect stimulated Ca^{2+} influx and neuroactive amino acid release from the slices. There were certain drugs which were more potent in affecting this $^{45}Ca^{2+}$ influx than others, but it was not possible to determine whether one receptor type was more effective than the others in mediating this effect because the variation in selectivity and potency of the agonists used in each group was greater than the variation between the receptor categories. However, amongst the μ-selective agonists, sufentanil was the most effective antagonist of Ca^{2+} entry and amino acid release from the cortical slices. At 1 μM the relative order of potency of the μ-selective agonists based on these parameters was sufentanil > fentanyl > etorphine > morphine (Table 1). The κ-selective agonists, tifluadom and U50,488H were approximately equipotent, whereas amongst the κ-selective agonists/μ-antagonists the relative order of potency at 10 μM was bremazocine > butorphenol > ethylketocyclazocine > nalbuphine > pentazocine.

These results are of particular interest when compared to those of Mulder *et al.* (1984) who found that μ-, κ- and σ-opioid receptor agonists differentially inhibited dopamine and acetylcholine release from rat striatal slices. Jones & Marchbanks (1982) observed that [D-Ala^2, Met^5]enkephalin inhibited stimulated noradrenaline release, but had no effect on acetylcholine release from cerebrocortical brain slices. We have found that none of the opioid receptor agonists used had differential effects on aspartate, glutamate or GABA release from cerebrocortical brain slices.

WIN44441-3 has proved to be stereospecific in its action in antagonizing opioid inhibition of veratrine-stimulated Ca^{2+} uptake and amino acid release in our experiments.

At high concentrations of fentanyl, tifluadom,

U50,488H, butorphanol and bremazocine there was a reduction in veratrine-induced release of K^+ from the slices. The opioid compounds may cause hyperpolarization of the membrane, so that it is less readily depolarized by veratrine (North & Williams, 1983). Any hyperpolarizing effect of the opioid compounds on the cerebrocortical slices would not necessarily be seen as a change of K^+ content, as the alteration of K^+ conductance may be too small to be detected, or may occur in a localized region which could be obscured by measurement of total K^+ content in the slices.

Many of the physiological effects of opioid compounds are likely to be due to an inhibition of neurotransmitter release. A reduction in transmitter release may be achieved by reducing the availability of free Ca^{2+} ions in the nerve terminal. Acute morphine treatment produces a selective decrease in both the Ca^{2+} content and the Ca^{2+} binding capacity of the synaptosomal fraction of brain tissue (Ross, 1977; Yamamoto *et al.*, 1978). Dunlap & Fischbach (1978) produced electrophysiological evidence that enkephalin acts directly on the voltage-dependent Ca^{2+} channels of dorsal root ganglion neurones grown in cell cultures. In our experiments opioid compounds appear to be acting like Ca^{2+} channel antagonists; inhibiting Ca^{2+} influx into cerebrocortical slices by a receptor-mediated mechanism. The effect of 100 μM morphine and 1 μM sufentanil on glutamate release appears to be much greater than 100%, i.e. the stimulated release of glutamate is completely blocked, and there is additionally an inhibition of spontaneous release. When added alone, 100 μM morphine and sufentanil had no effect on basal amino acid release. However, 100 μM morphine produced only 93% inhibition of stimulated aspartate release, and 105% inhibition of stimulated GABA release, therefore this result seems to be anomalous. Similarly, 1 μM sufentanil produced 83% inhibition of stimulated aspartate release and 95% inhibition of stimulated GABA release. For other opioid compounds, where inhibition appears to be greater than 100%, it can be taken as 100% inhibition within experimental variation.

In conclusion, it appears from this set of experiments that the μ- and κ-opioid receptors, and possibly the δ- and σ-opioid receptors, have a role to play in mediating an effect on calcium influx into cerebrocortical slices in a fashion which allows regulation of amino acid neurotransmitter release.

We are grateful for financial assistance from Sterling-Winthrop. E.J. White is in receipt of an MRC postgraduate studentship award. J.M. Crowder is in receipt of an SERC Quota Award. We thank Dr W. Schmidt (Du Pont, Delaware) for his helpful suggestions at the commencement of this project.

References

CHANG, K.J., COOPER, B.R., HAZUM, E. & CUATRECASAS, P. (1979). Multiple opiate receptors: different regional distribution in the brain and differential binding of opiates and opioid peptides. *Molec. Pharmac.* **16**, 91–104.

COTTON, R., GILES, M.G., MILLER, L., SHAW, J.S. & TIMMS, D. (1984). ICI 174864 – A highly selective antagonist for opioid δ receptor. *Eur. J. Pharmac.*, **97**, 331–332.

DHALIWAL, D. & BRADFORD, H.F. (1982). Inhibition of depolarization-coupled calcium fluxes and transmitter release *in vitro* by morphine. *FEBS Letts.*, **144**, 89–92.

DUNLAP, K. & FISCHBACH, G.D. (1978). Neurotransmitters decrease the calcium component of sensory neurone action potentials. *Nature*, **276**, 837–839.

GOLDSTEIN, A. & JAMES, I.F. (1984). Multiple opioid receptors: criteria for identification and classification. *Trends Pharmac. Sci.*, **5**, 503–505.

GOODMAN, R.R., SNYDER, S.H. (1982). κ-opiate receptors localized by autoradiography to deep layers of cerebral cortex: relation to sedative effects. *Proc. natn. Acad. Sci. U.S.A.*, **79**, 5703–5707.

JAMES, I.F. & GOLDSTEIN, A. (1984). Site-directed alkylation of multiple opioid receptors. *Molec. Pharmac.*, **25**, 337–342.

JONES, C.A. & MARCHBANKS, R.M. (1982). Effects of [D-alanine^2, methionine^5] enkephalinamide on the release of acetylcholine and noradrenaline from brain slices and isolated nerve terminals. *Biochem. Pharmac.*, **31**, 455–458.

LOWRY, O.H., ROSEBROUGH, G.H., FAIR, A.L. & RANDALL, R.J. (1951). Protein measurements with the folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

McILWAIN, H. (1961). Techniques in tissue metabolism. 5. Chopping and slicing tissue samples. *Biochem. J.*, **78**, 213–218.

MULDER, A.H., WARDEL, G., HOGENBOOM, F. & FRANKUYZEN, A.L. (1984). κ- and δ-opioid receptor agonists differentially inhibit striatal dopamine and acetylcholine release. *Nature*, **308**, 277–280.

NORTH, R.A. & WILLIAMS, J.T. (1983). How do opiates inhibit neurotransmitter release? *Trends Neurosci.* **6**, 337–339.

PATERSON, S.J., ROBSON, L.E. & KOSTERLITZ, H.W. (1983). Classification of opioid receptors. *Br. med. Bull.*, **39**, 31–36.

QUIRION, R., HAMMER, R., HERKENHAM, M. & PERT, C.B. (1981). Phencyclidine (angel dust)/δ-'opiate' receptor: visualization by tritium-sensitive film. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5881–5885.

ROMER, D., BUSCHLER, H., HILL, R.C., MAURER, R., PETCHER, T.J., WELLE, H.B.A., BAKEL, H.C.C.K. & AKKERMAN, A.M. (1980). Bremazocine: A potent, long-acting opiate kappa-agonist. *Life Sci.*, **27**, 971–978.

ROMER, D., BUSCHER, H.H., HILL, R.C., MAURER, R., PETCHER, T.J., ZEUGNER, H.., BENSON, W., FINNER, E., MILKOWSKI, W. & THIES, P.W. (1982). An opioid benzodiazepine. *Nature*, **298**, 759–760.

ROSENBAUM. J.S., HOLFORD, N.H.G., RICHARDS, M.L., AMAN, R.A. & SADEE, W. (1984). Discrimination of three

types of opioid binding sites in rat brain *in vivo*. *Molec. Pharmac.*, **25,** 242–248.

ROSS, D.H. (1977). Calcium content and binding in synaptosomes subfractions during chronic morphine treatment. *Neurochem. Res.*, **2,** 581–593.

TAKEMORI, A.E. & PORTUGHESE, P.S. (1984). Comparative antagonism by naltrexone and naloxone of μ, κ and δ agonists. *Eur. J. Pharmac.*, **104,** 101–104.

TAM, S.W. (1985). (+)-[^{3}H] SKF 10,047, (+)-[^{3}H] ethylketocyclazocine, μ, κ, δ and phencylclidine binding sites in guinea pig brain membranes. *Eur. J. Pharmac.*, **109,** 33–41.

VONVOIGTLANDER, P.F., LAHTI, R.A. & LUDENS, J.H. (1982). U50,488: A selective and structurally novel non-mu (kappa) opioid agonist. *J. Pharmac. exp. Ther.*, **244,** 7–12.

WARD, S.J., PIERSON, A.K. & MICHNE, W.F. (1983). Multiple opioid receptor profile *in vitro* and activity *in vivo* of the potent opioid antagonist WIN44441-3. *Life Sci.*, **33,** Supp. 1, 303–306.

WEDEGE, E., LUQMANI, Y. & BRADFORD, H.F. (1977). Stimulated incorporation of amino acids into proteins of synaptosomal fractions induced by depolarizing treatments. *J. Neurochem.*, **29,** 527–537.

YAMAMOTO, H., HARRIS, R.A., LOH, H.H. & WAY, E.L. (1978). Effects of acute and chronic morphine treatments on calcium localization and binding in brain. *J. Pharmac. exp. Ther.*, **205,** 255–264.

(*Received September 10, 1985.*
Revised January 17, 1986.
Accepted January 27, 1986.)