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INVESTIGATION OF ACTION OF ENKEPHALIN ON THE SPONTANEOUS AND EVOKED RELEASE OF ACETYLCHOLINE FROM RAT CORTICAL AND STRIATAL SLICES

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1 The effects of two synthetic enkephalins, D-Ala^2-Met^5-enkephalinamide (DALA) and D-Ala^2-MePhe^4-Met^5-(0)-ol-enkephalin (33,824) were tested on the spontaneous and stimulated release of acetylcholine (ACh) from rat cortical and striatal slices.

2 DALA, but not 33,824, caused a modest increase in spontaneous cortical ACh release. Neither enkephalin affected the release of cortical ACh induced by high potassium (25 mM) or veratridine (20 μM).

3 DALA had no effect on the release of ACh from striatal slices occurring spontaneously or in the presence of potassium (25 mM), veratridine (5 μM) or ouabain (10 μM). The enkephalin 33,824 also had no significant action on the striatal ACh release except that it caused a slight enhancement of veratridine-evoked release.

4 It is suggested that enkephalins have no significant action on release of ACh from the cortex or striatum *in vitro*. Their action on ACh release from the cortex *in vivo*, seen in previous studies, may be exerted at a subcortical level.

Introduction

Morphine and related opiate agonists depress the release of acetylcholine (ACh) from the cerebral cortex *in vivo*. Since this effect is stereospecific and can be blocked by the specific narcotic antagonist naloxone (Jhamandas, Phillis & Pinsky, 1971; Jhamandas, Hron & Sutak, 1975), it is mediated through opiate receptors. A unilateral application of morphine to the cortex produces a bilateral depression of ACh release (Jhamandas, Pinsky & Phillis, 1970); and lesioning of certain subcortical regions abolishes the inhibitory action of morphine on this release (Pepeu, Garau, Mulas & Marconcini-Pepeu, 1975; Jhamandas & Sutak, 1976). Therefore, these agents may be acting at a subcortical site to depress the *in vivo* release of ACh. *In vitro* studies on cortical slices have either failed to show an effect of opiates on ACh release (Szerb, 1974), or have demonstrated a depression which is not stereospecific and which occurs only with high doses of opiates (Sharkawi & Shulman, 1969; Howes, Harris & Dewey, 1970; Richter & Marchbanks, 1971; Jhamandas *et al.*, 1975). It seems therefore that the *in vitro* effect of opiate agonists on cortical ACh release is not mediated through opiate receptors.

We have shown that the endogenous opiate peptides methionine (Met) and leucine (Leu)-enkephalin also depress the *in vivo* release of cortical ACh, and that this effect is antagonized by naloxone (Jhamandas, Sawynok & Sutak, 1977). The effects of these peptides on the *in vitro* release of ACh specifically from the cerebral cortex have not been tested. Several investigations on morphine and enkephalins have shown that the opiate receptor population influenced by these agents is heterogeneous (Lord, Waterfield, Hughes & Kosterlitz, 1977; Lemaire, Magnan & Regoli, 1978; Schulz, Faase, Wüster & Herz, 1979; Chang & Cuatrecasas, 1979). A recent study from our laboratory which compared the action of morphine and several enkephalin analogues suggested that these agents were acting on different opiate receptors to influence the *in vivo* release of cortical ACh (Jhamandas & Sutak, 1980). It was also reported by others that the EEG and behavioural effects of morphine and enkephalins on the rat brain are mediated through different receptors (Tortella, Moreton & Khazan, 1978). It seems therefore that enkephalins may influence the *in vitro* release of ACh by activating an opiate receptor in the cortex which is not specifically sensitive to morphine.

Although the effects of enkephalins on the release of endogenous ACh from cortical slices have not been

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tested, there are two recent reports of opiate action on the release of ACh from slices of other brain regions. Met-enkephalin was reported to reduce the K^+-induced release of tritium (^3H) from rat hippocampal slices preloaded with [^3H]-choline (Subramanian, Mitznegg, Sprügel, Domschke, Domschke, Wünsch & Demling, 1977). Similarly, β-endorphin and D-Ala^2-Pro^5-enkephalinamide were found to enhance the ouabain-evoked release of ACh from rat striatal slices (Vizi, Hársing & Knoll, 1977). In the study described here, we have examined the action of two synthetic enkephalins on the spontaneous and stimulus-induced release of ACh from cerebral cortical slices. These synthetic enkephalins have been shown by us to depress cortical ACh release *in vivo* (Jhamandas & Sutak, 1980). For comparison, the effects of these enkephalins have also been examined on the release of ACh from striatal slices.

Methods

Release of acetylcholine

Male Sprague–Dawley rats, 200 to 300 g, were killed by decapitation. The brain was rapidly removed, placed on a cold glass plate, and cut in half along the midline. Without further dissection, two or three horizontal sections (0.5 mm thick) were sliced with a McIlwain tissue chopper. For striatal slices, the whole striatum was removed with forceps, and the tissue sliced (0.5 mm thick) on the chopper. Tissues were weighed (cortical slices: mean 73.5 $\pm$ 1.7 mg; striatal slices: mean 44.0 $\pm$ 0.9 mg) and placed in 2 ml Krebs solution containing physostigmine in a 10 ml beaker. The tissues were incubated in a Dubnoff shaking water bath at 37°C and gassed with 95% O_2 and 5% CO_2. Krebs solution contained (mM): NaCl 118, KCl 4.8, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2, $CaCl_2.2H_2O$ 2.5, glucose 11, $NaHCO_3$ 25 and physostigmine sulphate 7.7 μM.

The tissues were allowed to equilibrate for 30 min, during which time they were washed every 10 min by aspirating off the Krebs solution, and replacing it with fresh medium. Thereafter, ACh release was sampled in six 10 min collections as follows: four spontaneous release samples, one evoked release sample (in the presence of high K^+ or veratridine) and one recovery release sample. The tissues were washed once between the stimulated and recovery samples. When an enkephalin was tested, it was present in the third and fourth spontaneous release samples, and in the evoked release sample. Since ouabain takes longer to induce ACh release (Vizi *et al.*, 1977), its contact time with the tissue was two 10 min periods, and therefore 7 samples were collected.

Assay of acetylcholine

Acetylcholine released by the brain slices was assayed by the radioenzymatic method of Goldberg & McCaman (1974) as revised in our laboratory (Sawynok & Jhamandas, 1977), except that Amberlite CG-400 (Type II 200 mesh; BDH) was used instead of Dowex 1 × 8 for ion-exchange chromatography. The depolarizing agents and enkephalins used were tested for their interference with the ACh assay. The enkephalins, ouabain and veratridine did not interfere with this assay at the concentrations used in these experiments. A high concentration of K^+ (25 mM) does interfere with the assay but since the evoked-release samples were routinely diluted 1 in 5, and assayed in duplicate, this interference was avoided. The spontaneous release samples (0.5 ml) were assayed without dilution.

Drugs

D-Ala^2-Met^5-enkephalinamide (DALA) was obtained from Bachem Feinchemikalien (Bubendorf, Switzerland). D-Ala^2-MePhe^4-Met^5-(0)-ol-enkephalin (33,824) was a gift from Sandoz (Basel, Switzerland). Physostigmine (eserine) sulphate, ouabain octahydrate and veratridine were obtained from Sigma (St. Louis, Mo., USA).

Statistical tests

Student's unpaired t test was used to evaluate the significance of differences between experiments.

Results

The actual rates of spontaneous and stimulus-evoked release of ACh from isolated slices of the cerebral cortex and striatum, in control and enkephalin tests, are shown in Figures 1 to 4. To facilitate comparison of experiments which involved different combinations of depolarizing agents, enkephalins and brain regions, the results of various tests were standardized by expressing the release values as a percentage of the basal release of ACh. These data are shown in Table 1.

In control tests on cortical slices, the spontaneous release of ACh during the first two 10 min incubation periods (hereafter referred to as the basal release) occurred at a rate of about 200 pmol g^{-1} min^{-1} (Figures 1 and 2). This rate of spontaneous release was maintained during subsequent periods. High potassium concentration (25 mM), veratridine and ouabain were tested for their ability to stimulate ACh release. A 10 min application of potassium increased

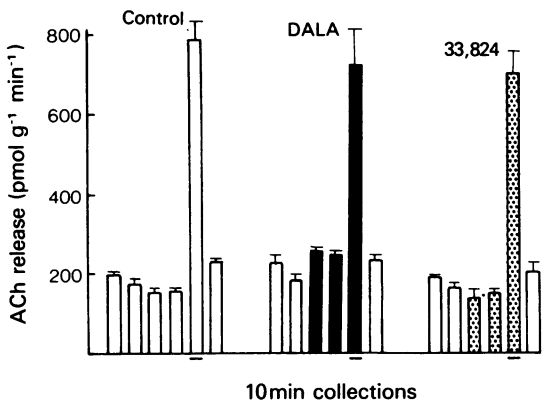

Figure 1 The effect of enkephalins D-Ala^2-Met^5-enkephalinamide (DALA) and D-Ala^2-MePhe^4-Met^5-(O)-ol-enkephalin (33,824) on the spontaneous and potassium (25 mM)-evoked release of acetylcholine (ACh) from cortical slices. The bars below the horizontal axis indicate the period of exposure to potassium. (Control $n = 4$; experimental $n = 8$; vertical lines show ± s.e. mean).

the release almost five fold; a more prolonged exposure did not produce a larger increase (data not shown). As shown in Figure 2 (insert), the alkaloid veratridine exerted a powerful, dose-related stimulatory action on cortical ACh release. Vizi *et al.* (1977) found that opiate peptides produce a two to three fold increase in the release of striatal ACh stimulated by ouabain. However, in our control tests, ouabain produced inconsistent changes in cortical ACh release; owing to this very large variability in the ouabain effect, the action of enkephalins could not be tested against ouabain-induced release. In subsequent tests, the effects of two enkephalins, DALA and 33,824 were tested on the spontaneous and stimulated release of cortical ACh. In two sets of experiments (Table 1), DALA produced a small but significant increase in the spontaneous ACh release, but 33,824 had no effect on this release. Both peptides failed to influence ACh release from cortical slices stimulated by potassium or veratridine.

In control experiments on striatal slices, the spontaneous release of ACh was about 500 pmol g^{-1} min^{-1}, a rate about 150% higher than that from corti-

Table 1 Effect of D-Ala^2-Met^5-enkephalinamide (DALA) and D-Ala^2-MePhe^4-Met^5-(0)-ol-enkephalin (33,824) on spontaneous and evoked acetylcholine (ACh) release from cortical and striatal slices.

	% *Basal ACh release** (× 100)							
	Control		*DALA*			*33,824*		
	Mean	s.e.	Mean	s.e.	*P*	Mean	s.e.	*P*
Cortical slices								
Potassium (25 mM)								
Spontaneous**	0.86[A]	0.08	1.22	0.11	<0.05	0.80	0.06	<0.6
Evoked***	4.40[A]	0.46	3.51	0.24	=0.10	4.10	0.51	<0.7
Veratridine (20 μM)								
Spontaneous	0.91	0.02	1.55	0.18	<0.01	0.87	0.05	<0.5
Evoked	7.19	0.74	8.74	0.74	<0.2	6.73	0.39	<0.6
Striatal slices								
Potassium (25 mM)								
Spontaneous	0.99	0.06	0.94	0.05	<0.6	0.82	0.09	<0.2
Evoked	8.35	1.27	8.45	0.63	>0.9	9.36	0.62	<0.5
Veratridine (5 μM)								
Spontaneous	0.91[B]	0.08	0.95	0.03	<0.6	0.85	0.03	<0.5
Evoked	8.58[B]	1.09	9.17	0.41	<0.6	11.67	0.55	<0.02
Ouabain (10 μM)								
Spontaneous	1.11	0.05	0.98	0.09	<0.3	0.88	0.05	<0.01
Evoked	14.49	1.75	14.51	1.26	>0.9	12.06[A]	0.90	<0.3

* Basal ACh release represents the average release during the first and second 10 min periods following the 30 min preincubation.

** Average release during third and fourth 10 min periods.

*** Release during the 10 min period of exposure to potassium or veratridine; in ouabain experiments the evoked release represents release occurring during the second 10 min exposure to this agent.

P values indicate significance of difference between release values in the absence and the presence of the enkephalin. $n = 8$ in all cases except A ($n = 7$) and B ($n = 4$).

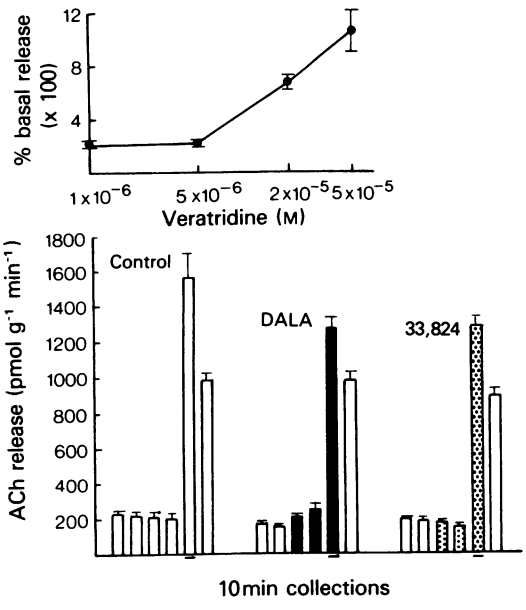

Figure 2 The effect of D-Ala^2-Met^5-enkephalinamide (DALA) and D-Ala^2-MePhe^4-Met-5(0)-ol-enkephalin (33,824) on the spontaneous and veratridine (20 μM)-evoked release of acetylcholine (ACh) from cortical slices. The bars below the horizontal axis indicate the period of exposure to veratridine (n = 8; vertical lines show s.e. mean). Insert: the effects of different veratridine concentrations on the release of ACh from cortical slices (n = 4).

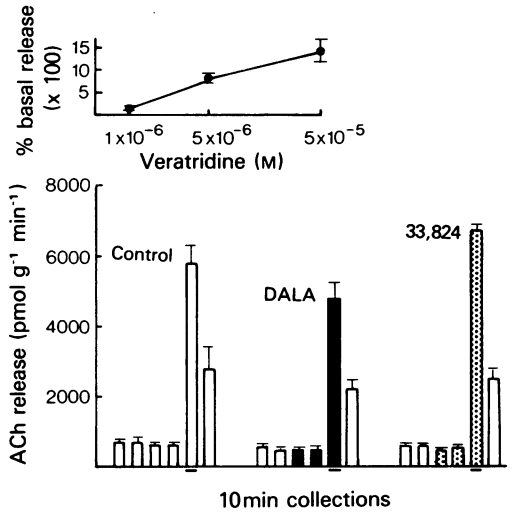

Figure 3 The effect of D-Ala^2-Met^5-enkephalinamide and D-Ala^2-MePhe^4-Met^5-(0)-ol-enkephalin (33,824) on the spontaneous and veratridine (5 μM)-evoked release of acetylcholine (ACh) from striatal slices. The bars below the horizontal axis indicate the period of exposure to veratridine. (Control n = 7; experimental n = 8; vertical lines show s.e. mean). Insert: the effects of different veratridine concentrations on the release of ACh from striatal slices (n = 4).

cal slices. This higher rate of release is consistent with the high content of ACh in the striatum. Potassium (25 mM) increased the striatal release of ACh about eight times. Veratridine produced a dose-related increase in the output of ACh from striatal slices, its effects in these tests generally exceeding those in the cortical experiments (Figure 3). In contrast to the cortical experiments, ouabain produced a large and consistent increase in the release of ACh from striatal slices (Figure 4). As shown previously by Vizi *et al.* (1977), the peak stimulatory effect of ouabain occurred in the second period following exposure to this agent; therefore in all tests in which ouabain was used, it was applied for two successive 10 min periods. In all tests on striatal slices, DALA did not significantly affect the spontaneous release of ACh. The enkephalin, 33,824, similarly produced very little change in this release, although a small decrease was seen on one occasion (Table 1). DALA also failed to influence the release of ACh evoked by the three depolarizing agents. The enkephalin, 33,824, had a variable effect on the evoked release: it slightly enhanced the release induced by veratridine, but did not significantly influence the release induced by other depolarizing agents (potassium or ouabain).

Discussion

If enkephalins depress the release of ACh by a direct action on the cholinergic nerve terminals in the central nervous system, then it should be possible to detect their action on this release in isolated slices. This study examined the effect of two enkephalins on the release of ACh occurring spontaneously and in response to different types of depolarizing stimuli (high potassium concentration, ouabain and veratridine) from the slices of cerebral cortex and striatum. The results of experiments on cortical slices show that enkephalins do not significantly inhibit the spontaneous or evoked release of ACh *in vitro*. This lack of inhibitory effect cannot be attributed to the metabolic instability of the enkephalins investigated here, since both DALA (Pert, Pert, Chang & Fong, 1976) and 33,824 (Roemer, Buescher, Hill, Pless, Bauer, Cardinaux, Closse, Hauser & Huguenin, 1977) are metabolically stable, and have been shown to

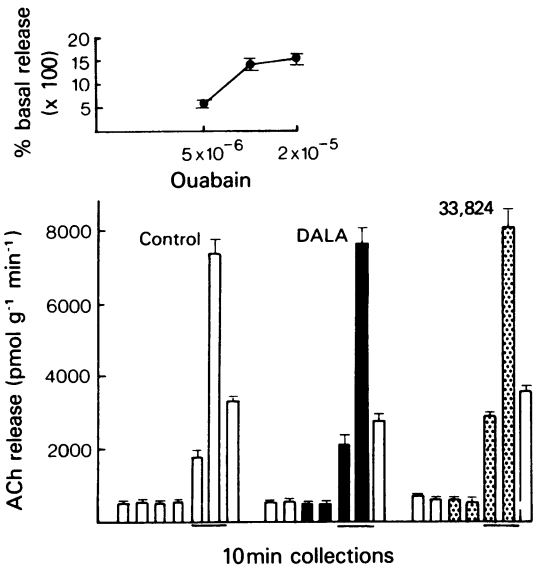

Figure 4 The effect of D-Ala^2-Met^5-enkephalinamide (DALA) and D-Ala^2-MePhe^4-Met^5-(O)-ol-enkephalin (33,824) on the spontaneous and ouabain (10 μM)-evoked release of acetylcholine (ACh) from striatal slices. The bars below the horizontal axis indicate the period of exposure to ouabain ($n = 8$; vertical lines show s.e. mean). Insert: the effects of different ouabain concentrations on the release of ACh from striatal slices. ($n = 4$).

depress cortical ACh release *in vivo* following intraventricular and systemic injection respectively (Jhamandas & Sutak, 1980). These findings tend to suggest that enkephalins do not act on cholinergic nerve terminals in the cortex, but that they might act primarily at a subcortical level to depress the release of ACh from the intact cerebral cortex. In tests on cortical slices, DALA enhanced the spontaneous release of ACh. However, since the other opiate peptide, 33,824, did not have this effect, the stimulatory effects of DALA on ACh may not involve opiate receptors.

The above observations on cortical ACh release are at variance with those of Subramanian *et al.* (1977), who showed that Met-enkephalin reduces the potassium evoked release of radioactivity from hippocampal slices preloaded with [^{3}H]-choline. There are two major differences between the latter study and the present investigation. The study by Subramanian *et al.* (1977) involved hippocampal slices, and the release of ACh in that study was measured as the ratio of total radioactivity (^{3}H) released into the medium to the total radioactivity present in the slice (the radioactivity in the slices would be associated with [^{3}H]-choline, [^{3}H]-ACh, and other [^{3}H]-choline derivatives). In our investigation the release of ACh was examined from cortical slices, and endogenous ACh was measured by a chemical assay. The discrepancy between the two studies might be related to the above differences. However, it should be pointed out that Szerb (1974) failed to observe an effect of morphine on the release of radioactivity from hippocampal slices preloaded with [^{3}H]-choline. Furthermore, it has been shown that lesions of the medial septum, which is the course of cholinergic nerve terminals to the hippocampus, do not change the binding of [^{3}H]-naloxone in the hippocampus (Kuhar, Pert & Snyder, 1973). This argues against the location of opiate receptors on the hippocampal cholinergic nerve terminals. Further work is warranted to resolve these differences.

Ouabain in our tests had an inconsistent effect on ACh release from cortical slices, although it consistently increased striatal release of this transmitter. The basis for a regional difference in the action of this glycoside is unclear. It may be related to the different characteristics of the cortical and striatal Na^+-K^+ ATPase, the inhibition of which is responsible for the action of ouabain; or it may be that in the cerebral cortex ouabain releases a substance that interferes with the release of ACh.

Vizi *et al.* (1977) have found that β-endorphin and D-Ala^2-Pro^5-enkephalinamide produce a powerful stimulatory action on the ouabain-evoked release of ACh from rat striatal slices; these effects were not observed in the present study. DALA and 33,824 generally failed to influence the striatal ACh release. The difference between the above findings and those of Vizi *et al.* (1977) is surprising, since both studies examined the endogenous release of ACh. In the latter study, the mass of slices used was variable (15 to 100 mg, less than 0.6 mm thick) and the ACh released in the incubation medium was assayed on the guinea-pig ileum. In the present investigation, the slice weight ranged between 34 and 52 mg (0.5 mm thick) and ACh was estimated by a chemical method. There is also a discrepancy concerning the action of morphine on ACh release from striatal slices: Szerb (1974) failed to observe an action of morphine on the release of radioactive ACh from rat striatal slices, while Vizi *et al.* (1977) found an increase in the release of endogenous ACh by morphine. These differences between various studies must be resolved before it can be concluded that enkephalins, or opiate analgesics, have a specific action on ACh release at the striatal level.

Conflicting results obtained between studies on the striatal slices could be partly related to the mass or thickness of slices used in release experiments. In most experimental studies, striatal slices include the caudate nucleus, putamen and the globus pallidus. However, Moroni, Cheney & Costa (1977) have found that β-endorphin inhibits ACh turnover in the rat

globus pallidus but not in the caudate nucleus; and Cuello & Paxinos (1978) showed that caudate nucleus sends enkephalinergic efferents to the globus pallidus. Therefore, opiate peptides may influence ACh release in the globus pallidus but not in the caudate. The successful demonstration of effects of enkephalins on striatal ACh release might therefore depend on the presence of pallidal neurones in these slices. It might be fruitful to examine the effects of peptides on ACh release from slices of the globus pallidus, and the caudate nucleus, in separate tests.

The absence of a clear inhibitory effect of the two synthetic enkephalins on the *in vitro* release of ACh from the cerebral cortex indicates that the depression of *in vivo* release, produced by these enkephalins in a previous study, is due to their action at a subcortical site.

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References

CHANG, K-J. & CUATRECASAS, P. (1979). Multiple opiate receptors. Enkephalins and morphine bind to receptors of different specificity. *J. biol. Chem.*, **254**, 2610–2618.

CUELLO, A.C. & PAXINOS, G. (1978). Evidence for a long Leu-enkephalin striopallidal pathway in rat brain. *Nature, Lond.*, **271**, 178–180.

GOLDBERG, A. M. & MCCAMAN, R.E. (1974). An enzymatic method for the determination of picomole amounts of choline and acetylcholine. In *Choline and Acetylcholine: Handbook of Chemical Assay Methods.* ed. Hanin, I. pp. 47–61. New York: Raven Press.

HOWES, J.F., HARRIS, L.S. & DEWEY, W.L. (1970). The effect of morphine, nalorphine, naloxone, pentazocine, cyclazocine and oxotremorine on the synthesis and release of acetylcholine by mouse cerebral cortex slices *in vitro. Archs int. Pharmacodyn.*, **184**, 267–276.

JHAMANDAS, K., HRON, V. & SUTAK, M. (1975). Comparative effects of opiate agonists methadone, levorphanol and their isomers on the release of cortical Ach *in vivo* and *in vitro. Can. J. Physiol. Pharmac.*, **53**, 540–548.

JHAMANDAS, K., PHILLIS, J.W. & PINSKY, C. (1971). Effects of narcotic analgesics and antagonists on the *in vivo* release of acetylcholine from the cerebral cortex of the cat. *Br. J. Pharmac.*, **43**, 53–66.

JHAMANDAS, K., PINSKY, C. & PHILLIS, J.W. (1970). Effects of morphine and its antagonists on release of cerebral cortical acetylcholine. *Nature, Lond.*, **228**, 176–177.

JHAMANDAS, K., SAWYNOK, J. & SUTAK, M. (1977). Enkephalin effects on release of brain acetylcholine. *Nature, Lond.*, **269**, 433–434.

JHAMANDAS, K. & SUTAK, M. (1976). Morphine-naloxone interaction in the central cholinergic system: the influence of subcortical lesioning and electrical stimulation. *Br. J. Pharmac.*, **58**, 101–107.

JHAMANDAS, K. & SUTAK, M. (1980). Action of enkephalin analogues and morphine on brain acetylcholine release: differential reversal by naloxone and an opiate pentapeptide. *Br. J. Pharm.*,

KUHAR, M.J., PERT, C.B. & SNYDER, S.H. (1973). Regional distribution of opiate receptor binding in monkey and human brain. *Nature, Lond.*, **245**, 447–450.

LEMAIRE, S., MAGNAN, J. & REGOLI, D. (1978). Rat vas deferens: a specific bioassay for endogenous opioid peptides. *Br. J. Pharmac.*, **64**, 327–329.

LORD, J.A.H., WATERFIELD, A.A., HUGHES, J. & KOSTERLITZ, H.W. (1977). Endogenous opioid peptides: multiple agonists and receptors. *Nature, Lond.*, **267**, 495–499.

MORONI, F., CHENEY, D.L. & COSTA, E. (1977). β-Endorphin inhibits ACh turnover in nuclei of rat brain. *Nature, Lond.*, **267**, 267–268.

PEPEU, G., GARAU, L., MULAS, M.L. & MARCONCINI-PEPEU, I. (1975). Stimulation by morphine of acetylcholine output from the cerebral cortex of septal rats. *Brain Res.*, **100**, 677–680.

PERT, C.B., PERT, A., CHANG, J-K. & FONG, B.T.W. (1976). [D-Ala^2]-Met-enkephalinamide: a potent, long-lasting synthetic pentapeptide analgesic. *Science*, **194**, 330–332.

RICHTER, J.A. & MARCHBANKS, R.M. (1971). Synthesis of radioactive acetylcholine from ^{3}H-choline and its release from cerebral cortex slices *in vitro. J. Neurochem.*, **18**, 691–703.

ROEMER, D., BUESCHER, H.H., HILL, R.C., PLESS, J., BAUER, W., CARDINAUX, F., CLOSSE, A., HAUSER, D. & HUGUENIN, R. (1977). A synthetic enkephalin analogue with prolonged parenteral and oral analgesic activity. *Nature, Lond.*, **268**, 547–549.

SAWYNOK, J. & JHAMANDAS, K. (1977). Muscarinic feedback inhibition of acetylcholine release from the myenteric plexus in the guinea-pig ileum and its status after chronic exposure to morphine. *Can. J. Physiol. Pharmac.*, **55**, 909–916.

SCHULZ, R., FAASE, E., WÜSTER, M. & HERZ, A. (1979). Selective receptors for β-endorphin on the rat vas deferens. *Life Sci.*, **24**, 843–850.

SHARKAWI, M. & SHULMAN, M.P. (1969). Inhibition by morphine of the release of ^{14}C-acetylcholine from rat brain cortical slices. *J. Pharm. Pharmac.*, **21**, 546–547.

SUBRAMANIAN, N., MITZNEGG, P., SPRÜGEL, W., DOMSCHKE, W., DOMSCHKE, S., WÜNSCH, E. & DEMLING, L. (1977). Influence of enkephalin on K^+-evoked efflux of putative neurotransmitters in rat brain. Selective inhibition of acetylcholine and dopamine release. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **299**, 163–165.

SZERB, J.C. (1974). Lack of effect of morphine in reducing the release of labelled acetylcholine from brain slices stimulated electrically. *Eur. J. Pharmac.*, **29**, 192–194.

TORTELLA, F.C., MORETON, J.E. & KHAZAN, N. (1978).

Electroencephalographic and behavioural effects of D-Ala^2-methionine-enkephalinamide and morphine in the rat. *J. Pharmac. exp. Ther.*, **206**, 636–643.

VIZI, E.S., HÁRSING, L.G. & KNOLL, J. (1977). Presynaptic inhibition leading to disinhibition of acetylcholine release from interneurons of the caudate nucleus: effects of dopamine, β-endorphin and D-Ala^2-Pro^5-enkephalinamide. *Neuroscience*, **2**, 953–961.