EFFECTS OF α-ENDORPHIN, β-ENDORPHIN AND (DES-TYR¹)-γ-ENDOR-PHIN ON α-MPT-INDUCED CATECHOLAMINE DISAPPEARANCE IN DISCRETE REGIONS OF THE RAT BRAIN

DIRK H. G. VERSTEEG, E. RONALD DE KLOET and DAVID DE WIED

Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Utrecht (The Netherlands)

SUMMARY

Following the intracerebroventricular administration of α-endorphin, β-endorphin and (des-tyrosine¹)-γ-endorphin in a dose of 100 ng, the α-MPT-induced catecholamine disappearance was found to be altered in discrete regions of the rat brain. In the regions in which α-endorphin exerted an effect, it without exception caused a decrease in catecholamine disappearance. Thus, in rats treated with α-endorphin the disappearance of noradrenaline was decreased in the medial septal nucleus, dorsomedial nucleus, central amygdaloid nucleus, subiculum, the ventral part of the nucleus reticularis medullae oblongatae and the A1 region, and that of dopamine in the caudate nucleus, globus pallidus, medial septal nucleus, nucleus interstitialis striae terminalis, paraventricular nucleus, zona incerta and central amygdaloid nucleus. β-Endorphin was found to decrease noradrenaline disappearance in the ventral part of the nucleus reticularis medullae oblongatae, dopamine disappearance in the lateral septal nucleus and the disappearance of both amines in the rostral part of the nucleus tractus solitarii. Dopamine disappearance was increased in the medial septal nucleus and the zona incerta following β-endorphin treatment. Following treatment with (des-tyrosine¹)-γ-endorphin, noradrenaline disappearance was enhanced in the anterior hypothalamic nucleus, whereas dopamine disappearance was increased in the paraventricular nucleus, the zona incerta and the rostral part of the nucleus tractus solitarii. In addition to this the latter peptide also caused a decreased noradrenaline disappearance in the periventricular thalamus and the A7 region. The results fit well with the suggestion that endorphins act as modulators of catecholamine neurotransmission in particular brain regions. The pattern of effects of the endorphins differ from that previously observed following intracerebroventricular administration of methionine-enkephalin. This is in keeping with the notion that the enkephalin containing network in the brain and that containing β-LPH represent two

independent systems with distinct differences in their projections to various brain regions.

INTRODUCTION

Although many observations indicate that the endorphins may act as modulators of neurotransmission in the brain, few data are available concerning the interaction of endorphins with catecholamine neurotransmission. In addition, the reports on effects of endorphins on catecholamine synthesis, turnover or release that have been published contain conflicting results. According to Izumi et al.[10], midbrain dopamine levels and turnover are decreased following the intracerebroventricular (i.c.v.) administration of β-endorphin (15 nmol/rat), whereas striatal dopamine is not significantly affected. Segal et al.[16] reported that, using a similar dose, β-endorphin does not alter caudate synaptosomal conversion of [^{3}H]tyrosine to [^{3}H]dopamine. Van Loon and Kim[20], on the other hand, observed increased DOPAC and HVA levels in rat striatum following β-endorphin administration, compatible with increased striatal dopamine turnover. Loh et al.[13] observed a decrease in potassium-induced [^{3}H]-dopamine release by striatal slices incubated with β-endorphin. In contrast, Arbilla and Langer[1] did not find an effect of β-endorphin on this parameter, whereas [^{3}H]noradrenaline release by cortical slices was diminished. Most of these discrepancies remain unexplained; possibly differences in methods and in doses might be factors contributing to some of them.

In this communication we present the results of a study in which the effects of low doses of β-LPH-fragments, administered i.c.v., were measured on the α-MPT-induced catecholamine disappearance in discrete regions of the rat brain. These fragments were α-endorphin (αE; β-LPH$_{61-76}$), β-endorphin (βE; β-LPH$_{61-91}$) and (des-tyr^1)-γ-endorphin (DTγE; β-LPH$_{62-77}$). The latter compound was included in this study, since it has been found to have effects opposite to αE and βE on the extinction of active avoidance behavior and the retention of passive avoidance behavior, whereas it is devoid of opiate-like activity[8].

METHODS

Male Wistar rats (130–150 g) were implanted with a polyethylene cannula in the lateral ventricle as described previously[4]. After a recovery period of 3 days the rats were subjected to the following treatment schedule. An i.p. injection with α-methyl-p-tyrosine methylester hydrochloride (α-MPT; Labkemi AB, Göteborg; 300 mg/kg) was followed, 30 min later, by the i.c.v. administration of either 1 μl saline or one of the following peptides: α-endorphin (αE; β-LPH$_{61-76}$), β-endorphin (βE; β-LPH$_{61-91}$) or (des-tyr^1)-γ-endorphin (DTγE; β-LPH$_{62-77}$), in a dose of 100 ng in 1 μl saline. Three hours after the saline or peptide was administered, i.e. 3.5 h after the injection of the tyrosine hydroxylase inhibitor α-MPT, the rats were killed. The brains were rapidly

taken out and frozen on dry ice. Subsequently, the brains were cut in 300 μm sections in a cryostat at —10 °C.

Brain regions and nuclei were dissected with hollow needles according to Palkovits[15] as described previously[18,23], except for the following regions.

Periventricular thalamus: this area was removed with a knife-cut from two sections at respectively 4500 and 4800 μm from the bregma at the midline thalamus level.

Zona incerta: the region extends from 1800 μm to 4200 μm and is located for the greater part between the lemniscus medialis and crus cerebri; punches of 500 μm diameter were removed from sections at 3900 and 4200 μm from the bregma.

Tissue of 2 rats was pooled. The tissue pellets were homogenized in 50 μl 0.1 N HClO$_4$. A 10 μl aliquot of the homogenate was taken for assay of the protein content[14]. The residual homogenate was centrifuged in the homogenization tubes (15 min; 15,000 $\times$ g; 4 °C). Noradrenaline and dopamine were assayed in a 20 μl sample of the supernatant as previously described[19]. Data are expressed in pg catecholamine per μg protein $\pm$ S.E.M. (n = 6–7). The significance of differences between groups was calculated using Student's t-test (two-tailed).

RESULTS

The results are summarized in Tables I and II. For all groups the catecholamine concentrations 3.5 h after inhibition of brain catecholamine synthesis with α-MPT are given. It should be realized that, since at the time of treatment with peptide or vehicle, i.e. 0.5 h after α-MPT administration, all tissues were at the same phase of catecholamine depletion, differences in catecholamine concentration 3 h later are a reflection of the effect of the peptides on α-MPT-induced catecholamine disappearance. Thus, the endpoint of the interaction of the peptides with the depletion process was determined (see also refs. 18 and 22).

Higher noradrenaline concentrations were found 3.5 h after α-MPT administration in the medial septal nucleus, the dorsomedial nucleus, the central amygdaloid nucleus, the subiculum, the ventral part of the nucleus reticularis medullae oblongatae and the A1 region of rats treated with αE than of those which had received saline; i.e. αE significantly decreased the noradrenaline disappearance induced by α-MPT in these brain regions (Table I). In the caudate nucleus, the globus pallidus, the medial septal nucleus, the nucleus interstitialis striae terminalis, the paraventricular nucleus, the zona incerta and the central amygdaloid nucleus αE caused a decrease in α-MPT-induced dopamine disappearance (Table II). βE was found to decrease noradrenaline disappearance in the ventral part of the nucleus reticularis medullae oblongatae and the rostral part of the nucleus tractus solitarii and dopamine disappearance in the lateral septal nucleus and the rostral part of the nucleus tractus solitarii. Dopamine disappearance was increased in the medial septal nucleus and the zona incerta of rats that had been treated with βE (Tables I and II). Following DTγE noradrenaline disappearance was increased in the anterior hypothalamic nucleus, whereas dopamine disappearance was increased in the paraventricular nucleus, the

TABLE I

Effect of α-endorphin, β-endorphin and DT-γ-endorphin on α-MPT-induced noradrenaline disappearance in discrete brain regions

Rats received α-MPT (300 mg/kg, i.p.) 3.5 h and, subsequently, saline or a β-LPH fragment (100 ng, dissolved in 1 μl saline, i.c.v.) 3 h prior to decapitation (for details, see text).

Brain regions	Noradrenaline (pg/μg protein)			
	Saline	α-endorphin (β-LPH$_{61-76}$)	β-endorphin (β-LPH$_{61-91}$)	DT-γ-endorphin (β-LPH$_{62-77}$)
Nucleus accumbens	4.9±0.6§	5.5±0.7	4.9±0.5	5.0±0.7
Caudate nucleus	n.d.§§	n.d.	n.d.	n.d.
Globus pallidus	7.9±1.1	7.1±1.0	5.6±0.8	6.4±0.7
Dorsal septal nucleus	4.7±0.7	6.0±0.9	5.7±0.5	6.3±0.9
Lateral septal nucleus	8.0±0.6	9.0±0.9	9.6±0.6	8.5±0.6
Medial septal nucleus	7.9±0.5	10.6±0.8**	6.7±1.4	9.2±1.0
Nucleus interstitialis striae terminalis	19.8±2.0	24.6±2.4	23.1±3.2	15.5±2.5
Supraoptic nucleus	9.3±1.0	11.3±1.4	7.9±0.8	10.4±1.3
Periventricular nucleus	20.6±1.6	23.3±2.3	23.7±3.1	25.5±3.1
Paraventricular nucleus	34.3±3.9	40.8±2.0	34.4±3.2	31.6±3.3
Anterior hypothalamic nucleus	12.1±0.8	11.2±0.7	11.7±1.5	8.6±0.7***
Arcuate nucleus	13.1±1.4	12.8±1.2	13.4±1.8	11.5±1.0
Median eminence	17.1±1.7	18.5±2.0	15.9±1.4	16.0±2.0
Zona incerta	4.4±0.3	4.7±0.5	4.2±0.4	3.8±0.3
Ventromedial nucleus	6.9±0.5	6.1±0.6	7.3±0.8	7.3±0.9
Dorsomedial nucleus	26.9±1.7	38.1±5.6**	27.5±3.9	24.2±2.7
Medial amygdaloid nucleus	3.0±0.4	3.8±0.5	3.3±0.4	3.9±0.5
Central amygdaloid nucleus	6.7±0.6	9.2±0.8*	8.5±0.8	6.8±0.6
Periventricular thalamus	4.5±0.1	4.9±0.3	5.3±0.6	6.5±0.8*
Parafascicular nucleus	5.2±0.3	4.9±0.5	5.4±0.6	5.5±0.7
Subiculum	0.7±0.06	0.9±0.08*	0.8±0.1	0.9±0.1
Gyrus dentatus	1.4±0.1	1.5±0.1	1.6±0.3	1.2±0.1
Nucleus raphe dorsalis	5.8±0.6	5.8±0.8	5.9±0.5	5.2±0.5
Nucleus ruber	1.6±0.2	1.2±0.2	1.7±0.3	1.5±0.2
Substantia grisea centralis dorsalis	1.7±0.2	1.8±0.2	1.8±0.2	1.7±0.2
Substantia grisea centralis ventralis	4.3±0.5	4.4±0.7	4.7±0.5	4.3±0.4
Colliculus inferior	1.6±0.2	1.4±0.1	1.6±0.2	1.7±0.2
Superior olive	2.6±0.2	2.2±0.2	2.3±0.2	2.1±0.2
Substantia nigra (reticularis)	1.9±0.2	1.8±0.2	1.9±0.3	2.0±0.5
A10 region	5.8±0.5	5.6±0.8	5.0±0.8	5.1±0.3
A9 region	4.7±0.6	4.3±0.6	4.3±0.7	4.5±0.3
A8 region	12.4±1.8	13.9±2.5	12.0±2.3	10.1±1.1
A7 region	6.0±0.4	6.6±0.9	5.5±0.7	9.0±1.0**
A6 region	15.9±2.2	16.3±1.7	12.4±1.7	12.3±1.2
A5 region	7.9±0.8	8.2±0.6	8.4±0.7	8.8±0.9
Nucleus parabrachialis dorsalis	4.6±0.3	4.3±0.2	4.3±0.3	4.2±0.4
Nucleus raphe magnus	5.4±0.8	5.0±0.5	6.2±0.6	5.2±0.5
Nucleus reticularis medullae oblongatae dorsalis	6.1±0.6	4.60±.6	6.0±0.8	5.8±0.9
Nucleus reticularis medullae oblongatae ventralis	4.3±0.3	6.1±0.7*	6.4±0.9*	5.3±0.7
A1 region	5.6±0.7	8.50±.4***	6.9±1.1	6.7±0.9
Nucleus tractus solitarii (rostralis)	6.0±0.6	6.8±1.1	8.0±0.5*	7.9±1.1
A2 region	22.2±2.5	25.7±3.1	26.0±3.8	27.1±2.8

§ Mean ± S.E.M. (n = 6–7).
§§ n.d. = not detectable.
* $P < 0.05$
** $P < 0.02$ for difference with saline treated controls (Student's t-test).
*** $P < 0.01$

TABLE II

Effect of α-endorphin, β-endorphin and DT-γ-endorphin on α-MPT-induced dopamine disappearance in discrete brain regions

Rats received α-MPT (300 mg/kg, i.p.) 3.5 h and, subsequently, saline or a β-LPH fragment (100 ng, dissolved in 1 μl saline, i.c.v.) 3 h prior to decapitation (for details, see text).

Brain regions	Dopamine (pg/μg protein)			
	Saline	α-endorphin (β-LPH$_{61-76}$)	β-endorphin (β-LPH$_{61-91}$)	DT-γ-endor-phin (β-LPH$_{62-77}$)
Nucleus accumbens	22.7±1.2§	21.9±1.7	25.5±0.9	23.8±1.5
Caudate nucleus	17.5±1.4	23.7±1.6**	20.5±0.7	18.9±2.0
Globus pallidus	3.2±0.5	4.9±0.5*	3.0±0.7	3.4±0.3
Dorsal septal nucleus	2.0±0.4	3.0±0.4	2.1±0.3	2.8±0.6
Lateral septal nucleus	4.3±0.5	5.0±0.6	6.9±0.5**	3.7±0.7
Medial septal nucleus	3.3±0.3	4.4±0.4*	1.9±0.5*	3.0±0.4
Nucleus interstitialis striae terminalis	3.1±0.4	4.9±0.4***	4.4±0.8	3.0±0.5
Supraoptic nucleus	n.d.§§	n.d.	n.d.	n.d.
Periventricular nucleus	2.2±0.4	1.8±0.2	1.9±0.3	2.4±0.4
Paraventricular nucleus	2.1±0.3	3.2±0.4*	1.7±0.3	1.3±0.2**
Anterior hypothalamic nucleus	1.3±0.3	1.2±0.2	1.3±0.3	1.9±0.4
Arcuate nucleus	3.0±0.6	2.8±0.7	4.0±0.6	2.6±0.4
Median eminence	12.5±1.4	11.1±1.6	12.5±2.1	11.5±0.8
Zona incerta	2.0±0.2	2.9±0.3*	0.9±0.1****	0.8±0.3***
Ventromedial nucleus	1.2±0.4	0.8±0.2	1.1±0.4	1.2±0.3
Dorsomedial nucleus	1.1±0.3	1.1±0.2	1.0±0.4	1.1±0.2
Medial amygdaloid nucleus	n.d.	n.d.	n.d.	n.d.
Central amygdaloid nucleus	2.0±0.3	3.6±0.6*	2.5±0.4	2.4±0.5
Periventricular thalamus	1.8±0.4	1.8±0.6	1.1±0.1	1.9±0.4
Parafascicular nucleus	n.d.	n.d.	n.d.	n.d.
Subiculum	n.d.	n.d.	n.d.	n.d.
Gyrus dentatus	n.d.	n.d.	n.d.	n.d.
Nucleus raphe dorsalis	2.0±0.3	2.5±0.3	3.0±0.4	2.2±0.3
Nucleus ruber	n.d.	n.d.	n.d.	n.d.
Substantia grisea centralis dorsalis	1.1±0.3	1.5±0.4	1.7±0.4	1.6±0.3
Substantia grisea centralis ventralis	0.6±0.1	0.9±0.2	1.0±0.3	0.7±0.2
Colliculus inferior	n.d.	n.d.	n.d.	n.d.
Superior olive	n.d.	n.d.	n.d.	n.d.
Substantia nigra (reticularis)	1.8±0.7	2.0±0.3	1.6±0.2	2.0±0.3
A10 region	4.1±0.8	3.5±0.7	3.1±0.5	2.9±0.3
A9 region	3.0±0.5	2.6±0.4	2.5±0.4	2.8±0.2
A8 region	1.6±0.3	1.6±0.3	1.2±0.2	1.4±0.2
A7 region	0.7±0.1	0.6±0.1	0.8±0.1	0.9±0.2
A6 region	n.d.	n.d.	n.d.	n.d.
A5 region	n.d.	n.d.	n.d.	n.d.
Nucleus parabrachialis dorsalis	1.1±0.4	1.2±0.5	1.1±0.2	1.1±0.2
Nucleus raphe magnus	0.9±0.3	0.9±0.2	0.6±0.2	0.9±0.2
Nucleus reticularis medullae oblongatae dorsalis	n.d.	n.d.	n.d.	n.d.
Nucleus reticularis medullae oblongatae ventralis	n.d.	n.d.	n.d.	n.d.
A1 region	n.d.	n.d.	n.d.	n.d.
Nucleus tractus solitarii (rostralis)	1.3±0.1	1.5±0.2	1.8±0.2*	0.7±0.1***
A2 region	1.1±0.2	0.9±0.1	1.0±0.3	1.0±0.4

§ Mean ± S.E.M. (n = 6–7).
§§ n.d. = not detectable.
* $P < 0.05$
** $P < 0.02$
*** $P < 0.01$
**** $P < 0.005$
for difference with saline treated controls (Student's t-test).

zona incerta and the rostral part of the nucleus tractus solitarii (Tables I and II). In addition to this DTγE also was found to cause a decreased noradrenaline disappearance in the periventricular thalamus and the A7 region (Table I).

DISCUSSION

From the results it is clear that endorphins, administered i.c.v. in a low dose, exert pronounced effects on the regional α-MPT-induced disappearance of noradrenaline and, particularly, dopamine in the brain. In similar studies[18,22] we observed effects of i.c.v., vasopressin and methionine-enkephalin on catecholamine metabolism in discrete brain regions using doses as low as 100 ng. Nevertheless, these doses, albeit low, were 10–50 times higher than those found to exert effects on avoidance behavior[4,7]. Also the dose of 100 ng per rat i.c.v. employed in the present study for the endorphins was based on the finding that effects of these peptides on avoidance behavior are evident following their i.c.v. administration in doses as low as 0.3–3 ng (refs. 7, 8).

Effects were particularly abundant following αE administration. Without exception this peptide caused a decrease in α-MPT-induced catecholamine disappearance in those regions that were sensitive to this neuropeptide. The effects of βE and the des-tyr[1] analog of γE were not as widespread as those of αE. Moreover, in contrast to αE, in some regions βE and DTγE caused an acceleration in catecholamine disappearance following synthesis inhibition. The regions where effects were observed in this study in many cases are rich in β-LPH and/or enkephalins[2,9,16,17,24,25]. This is in agreement with the supposition that the observed effects are the result of an interaction of the administered peptides with receptors involved in the modulation of catecholamine neurotransmission by neuronal systems containing β-LPH fragments. It should be noted that the pattern of effects of the endorphins is clearly different from that previously observed for methionine-enkephalin[22]. In contrast to methionine-enkephalin the endorphins failed to affect catecholamine disappearance in the central gray (cf. ref. 22). This difference in pattern, however, is in keeping with the idea that the enkephalin containing network in the brain and that containing β-LPH represent two independent systems with distinct differences in their projections to various brain regions[2,9,16,24,25].

As is summarized in the Introduction, most literature data concern effects, or absence of effects, only of βE in much higher doses than used in the present study on striatal dopamine metabolism[1,10,13,16,20]. From the present results it appears that the pattern of effects of αE and βE are rather different. For example, whereas αE caused a decrease in α-MPT-induced dopamine disappearance in the caudate nucleus and the globus pallidus, but not in the nucleus accumbens, βE was without effect in either of these structures, though there was a tendency towards a decrease in the caudate nucleus. DTγE, which in contrast to αE facilitates the extinction of active avoidance behavior and attenuates passive avoidance behavior[8], affected dopamine disappearance in the paraventricular nucleus and the zona incerta in a direction opposite to that induced by αE. γE and its des-tyrosine[1] analog DTγE have similar effects on avoidance

behavior; removal of the N-terminal tyrosine residue of γE, as is the case in DTγE, however, yields a peptide that is more potent on avoidance behavior but lacks opiate-like activity[8]. Differences in the intracerebral degradation of the three peptides might be a factor in the observed dissociation of effects. Functional endorphin metabolism may occur in the brain: αE, γE and DTγE and other, as yet unidentified peptides, have been found to accumulate when βE is incubated in vitro with synaptosomal membranes from brain tissue (see ref. 3 and Burbach et al., to be published). It is conceivable that, as a result of regional differences in peptidase activities, further information, concealed in the structure of βE, can come to light.

Several lines of evidence have provided a basis for the postulate that normal functioning of the brain would require a balance between DTγE or related peptides and α-type endorphins[5,8,21]. Schizophrenia, according to this postulate, is the result of a relative excess of α-type endorphins in the brain[5,21]. On basis of its effects on conditioned avoidance behavior it has been suggested that DTγE is an endogenous peptide with neuroleptic-like activity with a profile more specific than that of currently used neuroleptics[5,6,8,21]. This latter suggestion was made since, in contrast to neuroleptics like haloperidol, DTγE did not cause sedation nor did it affect locomotion[8,11]. Haloperidol has been shown to enhance striatal dopamine release[12,16]. DTγE, however, did not cause changes in dopamine disappearance in the nigrostriatal system. It is tempting to speculate that the neuroleptic-like effects of DTγE are the result of an interaction of the peptide with other dopaminergic systems than the nigro-striatal system. Further experiments should help to test this possibility and, also, to elucidate what might be the mechanism of action of the endorphins.

ACKNOWLEDGEMENTS

REFERENCES

1 Arbilla, S. and Langer, S. Z., Morphine and β-endorphin inhibit release of noradrenaline from cerebral cortex but not of dopamine from rat striatum, *Nature (Lond.)*, 271 (1978) 559–561.

2 Barchas, J. D., Akil, H., Elliott, G. R., Holman, R. B. and Watson, S. J., Behavioral neurochemistry: neuroregulators and behavioral states, *Science*, 200 (1978) 964–973.

3 Burbach, J. P. H., Loeber, J. G., Verhoef, J., De Kloet, E. R. and De Wied, D., Biotransformation of endorphins by a synaptosomal membrane preparation of rat brain and by human serum, *Biochem. biophys. Res. Commun.*, 86 (1979) 1296–1303.

4 De Wied, D., Behavioral effects of intraventricularly administered vasopressin and vasopressin fragments, *Life Sci.*, 19 (1976) 685–690.

5 De Wied, D., Psychopathology as a neuropeptide dysfunction. In J. M. Van Ree and L. Terenius (Eds.), *Characteristics and Function of Opioids*, Elsevier/North-Holland Biomedical Press, Amsterdam, 1978, pp. 113–122.

6 De Wied, D., Bohus, B., Van Ree, J. M., Kovács, G. L. and Greven ,H. M., Neuroleptic-like activity of (des-tyr^1)-γ-endorphin in rats, *Lancet*, I (1978) 1046.

7 De Wied, D., Bohus, H., Van Ree, J. M. and Urban, I., Behavioral and electrophysiological effects of peptides related to lipotropin (β-LPH), *J. Pharmacol. exp. Ther.*, 204 (1978) 570–580.

8 De Wied, D., Kovács, G. L., Bohus, B., Van Ree, J. M. and Greven, H. M., Neuroleptic activity of the neuropeptide β-LPH 62–77 ((des-tyr^1)-γ-endorphin; DTγE), *Europ. J. Pharmacol.*, 49 (1978) 427–436.

9 Elde, R., Hökfelt, T., Johansson, O. and Terenius, L., Immunohistochemical studies using antibodies to leucine enkephalin. Initial observations on the nervous system of the rat, *Neurosci.*, 1 (1976) 349–351.

10 Izumi, K., Motomatsu, T., Chrétien, M., Butterworth, R. F., Lis, M., Seidah, N. and Barbeau, A., β-Endorphin induced akinesia in rats. Effect of apomorphine and α-methyl-p-tyrosine and related modifications of dopamine turnover in the basal ganglia, *Life Sci.*, 20 (1977) 1149–1156.

11 Kovács, G. K. and De Wied, D., Effects of amphetamine and haloperidol on avoidance behavior and exploratory activity, *Europ. J. Pharmacol.*, 53 (1978) 103–107.

12 Kuczenski, R., Schmidt, D. and Leith, N., Amphetamine–haloperidol interactions in rat striatum: failure to correlate behavioral effects with dopaminergic and cholinergic dynamics, *Brain Research*, 126 (1977) 117–129.

13 Loh, H. H., Brase, D. A., Sampath-Khanna, S., Mar, J. B., Way, E. L. and Li, C. H., β-Endorphin in vitro inhibition of striatal dopamine release, *Nature (Lond.)*, 264 (1976) 567–568.

14 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with Folin phenol reagent, *J. biol. Chem.*, 193 (1951) 265–275.

15 Palkovits, M., Isolated removal of hypothalamic or other brain nuclei of the rat, *Brain Research*, 59 (1973) 449–450.

16 Segal, D. S., Browne, R. G., Bloom, F., Ling, N. and Guillemin, R., β-Endorphin: endogenous opiate or neuroleptic?, *Science*, 198 (1977) 411–414.

17 Simantov, R., Kuhar, M. J., Uhl, G. R. and Snyder, S. H., Opioid peptide enkephalin: immuno-histochemical mapping in rat central nervous system, *Proc. nat. Acad. Sci. (Wash.)*, 74 (1977) 2167–2171.

18 Tanaka, M., De Kloet, E. R., De Wied, D. and Versteeg, D. H. G., Arginine^8-vasopressin affects catecholamine metabolism in specific brain nuclei, *Life Sci.*, 20 (1977) 1799–1808.

19 Van Der Gugten, J., Palkovits, M., Wijnen, H. J. L. M. and Versteeg, D. H. G., Regional distribution of adrenaline in rat brain, *Brain Research*, 107 (1976) 171–175.

20 Van Loon, G. R. and Kim, C., Effect of β-endorphin on striatal dopamine metabolism, *Res. Commun. chem. path. Pharmacol.*, 18 (1977) 171–174.

21 Van Ree, J. M., Verhoeven, W. M. A., Van Praag, H. M. and De Wied, D., Antipsychotic action of (des-tyr^1)-β-endorphin (β-LPH$_{62-77}$), In J. M. Van Ree and L. Terenius (Eds.), *Characteristics and Function of Opioids*, Elsevier/North-Holland Biomedical Press, Amsterdam, 1978, pp. 181–184.

22 Versteeg, D. H. G., De Kloet, E. R. and De Wied, D., Interaction of endorphins with brain catecholamine systems, In J. M. Van Ree and L. Terenius (Eds.), *Characteristics and Function of Opioids*, Elsevier/North-Holland Biomedical Press, Amsterdam, 1978, pp. 323–331.

23 Versteeg, D. H. G., Van der Gugten, J., De Jong, W. and Palkovits, M., Regional concentrations of noradrenaline and dopamine in rat brain, *Brain Research*, 113 (1976) 563–574.

24 Watson, S. J., Akil, H., Sullivan, S. and Barchas, J. D., Immunocytochemical localization of methionine enkephalin: Preliminary observations, *Life Sci.*, 21 (1977) 733–738.

25 Watson, S. J., Barchas, J. D. and Li, C. H., β-Lipotropin: Localization of cells and axons in rat brain by immunocytochemistry, *Proc. nat. Acad. Sci. (Wash.)*, 74 (1977) 5155–5158.