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Pro-Leu-GlyNH_2 Affects Dopamine and Noradrenaline Utilization in Rat Limbic-Forebrain Nuclei

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The effects of Pro-Leu-GlyNH_2 (PLG), administered i.c.v. in doses of 3.5, 35, 350 and 3500 pmol, were studied on the α-MPT-induced disappearance of catecholamines in microdissected rat brain nuclei. PLG, dose-dependently, increased dopamine disappearance in the nucleus caudatus and globus pallidus, whereas a decrease in dopamine disappearance was observed in the nucleus dorsomedialis. Noradrenaline disappearance was decreased in the medial septal nucleus, anterior hypothalamic area and lateral amygdala. A tendency towards an increase in noradrenaline disappearance was observed in the nucl. supraopticus. These data show that PLG has a central site of action. The effects of PLG on dopamine disappearance are comparable to those previously found with vasopressin, while the effects of PLG on noradrenaline utilization show a striking similarity with those previously obtained with oxytocin.

INTRODUCTION

The neuropeptide, Pro-Leu-GlyNH_2 (PLG), was first isolated from bovine hypothalamic tissue by Nair et al.[28], as a peptide with MSH-release-inhibiting activity. PLG has since then been the subject of numerous behavioural, neuropharmacological, biochemical and clinical studies[19,20,21,43]. The results of many of these studies have suggested that PLG interacts with central dopaminergic systems. However, the available biochemical evidence is not unanimous in support for this suggestion. In most cases only the interaction of PLG with the nigrostriatal dopamine system was investigated. Positive results have been reported on dopamine concentration[17], in vitro dopamine synthesis[17], tyrosine hydroxylase activity[33] and the α-MPT-induced disappearance of dopamine[33,44]. On the other hand, also negative reports have been published concerning the effects of PLG on these parameters[22–24,31]. Several factors have to be considered with respect to these conflicting results. These include the dose level, the fact whether an acute or chronic treatment was used, the route of administration (central or peripheral), single dose or dose–response curves, the parameter being measured, the treatment schedule, the sensitivity of the rat strain and the pre-experimental handling.

Up until now little is known concerning an interaction of PLG with other catecholamine-containing neurons in the brain. Recently it was found that PLG also had an effect on hippocampal and hypothalamic noradrenaline-containing neurons[34]. Therefore in the present study the effect of PLG was studied on central dopaminergic and noradrenergic activity more in detail, by examining the effect of PLG after i.c.v. injection in several doses on the α-MPT-induced disappearance of dopamine and noradrenaline in several microdissected brain nuclei.

METHODS

Male Wistar rats (140–160 g) were used. A polyethylene canula was implanted in the lateral ventricle as described previously[10]. After 5 days of recovery and handling, the rats were subjected to the following treatment schedule. An i.p. injection with

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α-methyl-*p*-tyrosine methylester HCl (α-MPT; Labkemi AB, Göteborg, Sweden; 300 mg/kg) was, followed, 30 min later by an i.c.v. injection of 1 μl saline or PLG in doses of 3.5, 35, 350 and 3500 pmol in 1 μl saline. Three hours after the saline or peptide was administered the rats were decapitated. The brains were rapidly taken out of the skull and frozen on dry-ice. The brains were stored at −80 °C. The brains were cut in 300 μm sections in a cryostat at −10 °C. Nuclei were punched according to the microdissection method described by Palkovits[29]. The tissue pellets were homogenized in 70 μl 0.1 N $HClO_4$. The homogenate was centrifuged (15 min, 15,000 *g*, 4 °C). Noradrenaline and dopamine were assayed in a 20 μl sample of the supernatant as described previously[36]. The pellet was redissolved in 1.1 N NaOH and an aliquot was taken for protein assay[25]. Data were calculated as pg catecholamine per μg protein ± S.E.M. Two experiments were performed. In the first experiment the brain nuclei of individual rats were measured. In the second experiment brain nuclei of two rats were pooled. The results of both experiments are combined. The significance of the differences was analyzed using one-way ANOVA and Student's *t*-test (two-tailed).

RESULTS

The data of both experiments are combined and the results are summarized in Tables I and II. As can be seen, i.c.v. injection of PLG resulted in a change in the α-MPT-induced disappearance of dopamine and noradrenaline in only a few brain nuclei. PLG enhanced the utilization of dopamine in the nucl. caudatus at doses of 3.5, 35 and 350 pmol and in the globus pallidus at a dose of 3.5 pmol. In the nucl. dorsomedialis the dopamine utilization was inhibited at doses of 3.5 and 35 pmol. Noradrenaline utilization was significantly decreased in the medial septal nucleus at doses of 3.5 and 35 pmol and in the lateral amygdala at doses of 3.5 and 35 pmol. A tendency towards a change in noradrenaline utilization was found in the nucl. supraopticus, where PLG increased noradrenaline utilization at a dose of 3.5 pmol and in the anterior hypothalamic area where PLG decreased the noradrenaline utilization at doses of 3.5, 35 and 350 pmol.

DISCUSSION

The present results show that i.c.v. administered

TABLE I

Effect of Pro-Leu-GlyNH_2 on dopamine concentrations in discrete brain nuclei, 3.5 h after α-MPT injection

Rats received α-MPT (300 mg/kg, i.p.) 3.5 h and subsequently saline or peptide at doses indicated in the table, 3 h prior to decapitation. For further details see text. ANOVA for dopamine in the nucl. caudatus $F(4,103) = 2.55$, $P < 0.05$; globus pallidus $F(4,30) = 3.18$, $P < 0.05$; nucl. dorsomedialis $F(4,36) = 4.56$, $P < 0.05$. Mean ± S.E.M. are given, n = 6–9 or 15–18.

Brain nucleus	*Dopamine (pg/μg protein)*				
	Saline	*Pro-Leu-GlyNH_2 (pmol)*			
		3.5	*35*	*350*	*3500*
Nucl. accumbens	24.1 ± 1.5	24.1 ± 1.5	23.5 ± 1.9	24.4 ± 2.8	24.0 ± 1.6
Nucl. caudatus	32.2 ± 1.9	27.0 ± 1.1*	27.7 ± 1.4*	27.1 ± 1.1*	29.1 ± 1.7
Dorsal septal nucleus	2.90 ± 0.30	2.80 ± 0.22	3.04 ± 0.23	2.90 ± 0.25	3.86 ± 0.66
Lateral septal nucleus	9.45 ± 1.06	9.63 ± 0.93	9.35 ± 0.95	12.97 ± 1.58	10.69 ± 1.98
Medial septal nucleus	2.12 ± 0.26	2.60 ± 0.39	2.56 ± 0.35	2.25 ± 0.24	2.43 ± 0.44
Nucl. supraopticus	3.51 ± 0.29	3.42 ± 0.40	3.51 ± 0.47	3.25 ± 0.23	
Nucl. paraventricularis	1.08 ± 0.21	0.99 ± 0.06	1.16 ± 0.21	1.21 ± 0.31	0.88 ± 0.16
Anterior hypothalamic area	1.08 ± 0.10	1.16 ± 0.08	1.22 ± 0.10	1.37 ± 0.16	0.88 ± 0.16
Globus pallidus	1.22 ± 0.25	0.47 ± 0.08*	1.17 ± 0.24	0.90 ± 0.11	1.17 ± 0.22
Nucleus dorsomedialis	0.68 ± 0.08	1.08 ± 0.12*	1.24 ± 0.12*	0.73 ± 0.12	0.97 ± 0.12
Medial amygdala	n.d.	n.d.	n.d.	n.d.	n.d.
Central amygdala	0.80 ± 0.11	0.72 ± 0.06	0.54 ± 0.09	0.62 ± 0.15	0.88 ± 0.16
Lateral amygdala	1.05 ± 0.06	0.99 ± 0.07	1.01 ± 0.05	0.96 ± 0.06	1.07 ± 0.09
Gyrus dentatus	0.38 ± 0.10	0.73 ± 0.14	0.47 ± 0.10	0.41 ± 0.08	0.45 ± 0.07
Nucleus parafascicularis	0.27 ± 0.03	0.26 ± 0.07	0.35 ± 0.06	0.30 ± 0.06	0.28 ± 0.02

* $P < 0.05$ Student's *t*-test.

TABLE II

Effect of Pro-Leu-GlyNH_2 on the noradrenaline concentrations in discrete brain nuclei, 3.5 h after α-MPT injection

Rats received α-MPT (300 mg/kg, i.p.) 3.5 h and subsequently saline or peptide at doses indicated in the table, 3 h prior to decapitation. For further details see text. ANOVA for noradrenaline in the medial septal nucleus $F(4,67) = 2.95$, $P < 0.05$; nucleus supraopticus $F(4,57) = 1.94$, $P < 0.1$; anterior hypothalamic area $F(4,64) = 1.60$, $P > 0.1$; lateral amygdala $F(4,74) = 4.14$, $P < 0.05$. Mean ± S.E.M. are given, n = 6–9 or 15–18.

Brain nucleus	*Noradrenaline (pg/μg protein)*				
	Saline	*Pro-Leu-GlyNH_2 (pmol)*			
		3.5	*35*	*350*	*3500*
Nucl. accumbens	1.08 ± 0.07	1.16 ± 0.06	1.26 ± 0.04	1.33 ± 0.14	1.20 ± 0.03
Nucl. caudatus	n.d.	n.d.	n.d.	n.d.	n.d.
Dorsal septal nucleus	2.90 ± 0.22	2.80 ± 0.30	3.04 ± 0.23	2.90 ± 0.25	3.86 ± 0.66
Lateral septal nucleus	7.34 ± 0.37	8.18 ± 0.52	8.78 ± 0.71	7.61 ± 0.41	7.74 ± 1.29
Medial septal nucleus	8.62 ± 0.67	11.44 ± 1.24*	10.96 ± 0.72*	9.79 ± 0.65	8.06 ± 0.67
Nucl. supraopticus	8.45 ± 1.03	6.01 ± 0.68*	7.28 ± 0.86	9.08 ± 0.91	6.67 ± 0.74
Nucl. paraventricularis	22.91 ± 3.41	22.23 ± 3.39	26.34 ± 4.15	20.98 ± 1.53	28.85 ± 5.29
Anterior hypothalmic area	5.58 ± 0.41	6.43 ± 0.48*	6.52 ± 0.43*	7.81 ± 1.11*	5.82 ± 0.45
Globus pallidus	4.06 ± 0.70	3.66 ± 1.01	3.43 ± 0.81	3.46 ± 0.76	3.00 ± 0.33
Nucl. dorsomedialis	41.87 ± 4.73	50.95 ± 6.11	46.73 ± 5.73	43.20 ± 6.10	42.37 ± 4.96
Medial amygdala	3.85 ± 0.57	3.06 ± 0.50	3.42 ± 0.68	3.94 ± 0.91	3.30 ± 0.30
Central amygdala	1.04 ± 0.07	0.87 ± 0.09	0.91 ± 0.13	1.11 ± 0.16	1.05 ± 0.16
Lateral amygdala	5.55 ± 0.35	7.23 ± 0.44*	6.64 ± 0.36*	5.45 ± 0.39	6.78 ± 0.44
Gyrus dentatus	6.61 ± 0.59	6.99 ± 1.23	7.03 ± 0.55	7.13 ± 1.27	7.61 ± 1.20
Nucl. parafascicularis	1.00 ± 0.13	1.00 ± 0.07	0.98 ± 0.07	0.85 ± 0.10	1.00 ± 0.06

* $P < 0.05$ Student's *t*-test.

PLG elicits changes in catecholamine utilization in a limited number of brain nuclei of the rat. The dose–response curves observed for the effect of PLG on dopamine utilization in the nucl. caudatus, globus pallidus and nucl. dorsomedialis and on noradrenaline utilization in the medial septal nucleus and the lateral amygdala are non-linear, bell-shaped in all cases: effects were evident after the lower doses (3.5, 35 and 350 pmol) only and not after the higher dose (3500 pmol). In a comparable study in which the effects of i.c.v. administered PLG were measured on catecholamine utilization in large brain parts, Szabó et al.[34] also found bell-shaped dose–response curves on dopamine utilization in the striatum and on noradrenaline utilization in the hypothalamus and hippocampus.

Most biochemical studies with PLG have focussed on the effect of the peptide on various parameters of striatal dopaminergic activity. Table III summarizes these literature data. From these data it appears that PLG is not effective in altering dopaminergic activity when given in high doses. In addition to these biochemical findings there are the results of clinical studies in which PLG was administered to depressive patients[13,14] and to patients suffering from Parkinson's disease[3] and tardive dyskinesia[15]. Also these results show that the effects of PLG do not increase with the dose level, but follow an inverted-U-shaped curve. Bell-shaped dose–response curves have also been reported on the release of ACTH from dispersed pituitary cells[45], on DOPA potentiation and fluphenazin antagonism[46], on morphine-induced catalepsy[6] and on neuroleptic-induced catalepsy and dopamine/neuroleptic receptor binding[7]. Data concerning the uptake and metabolism of s.c.[41] and i.v. and i.c.v.[11] administered [^{3}H]PLG support the view that systemic doses of PLG in the high mg range lead to brain concentrations of intact PLG exceeding those which appear to be effective in altering catecholamine utilization following i.c.v. administration of the peptide as shown in the present study and by Szabó et al.[34]. Together these data might explain why conflicting data have been obtained in various studies on effects of PLG on striatal dopaminergic activity (for references see legend Table III), specifically in single-dose studies.

The present results show that in general PLG decreases noradrenaline utilization in a number of lim-

TABLE III

Effect of Pro-Leu-GlyNH$_2$ (PLG) on striatal dopamine neurotransmission in the rat brain

Treatment	*Dose*	*Method*	*Effect*	*Ref.*
Systemic	1 mg/kg	DOPAC and HVA concentr.	0	22
	0.5, 1 and 5 mg/kg	in vitro [^{3}H]DA synthesis	increase	17
	3 mg/kg	TH activity	0	33
	3 mg/kg	DA and HVA concentr.	0	33
	5 mg/kg	TH activity	increase	33
	5 mg/kg	DA and HVA concentr.	increase	33
	20 mg/kg	DA concentr.	0	23
	10, 30 and 100 mg/kg	DOPA accumulation	0	1
	100 mg/kg	DA and HVA concentr.	0	31
i.c.v.	1 and 10 ng/rat*	DA concentr.	increase	34
	1 and 10 ng/rat*	DA utilization (α-MPT)	increase	34
	1, 10 and 100 ng/rat*	DA utilization(α-MPT)	increase	present results
	200 ng/rat	DA utilization(α-MPT)	increase	44
	200 ng/rat	DA utilization(α-MPT)	0	22

* Higher doses were not effective.

bic-forebrain nuclei, with the exception of the nucl. supraopticus, where a tendency towards an increasing effect was found. The dose–response curves are, again, bell-shaped. Decreasing effects of PLG on noradrenaline utilization have also been reported in the hypothalamus and hippocampus[34]. Comparing the present results with those previously obtained with the two related peptides, vasopressin and oxytocin, some interesting similarities and differences emerge. The effects of PLG on dopamine utilization resemble those which have been observed previously with vasopressin[35]. The effects of PLG on noradrenaline utilization show a striking similarity with those previously obtained with oxytocin[38]. In general the effects of PLG and oxytocin on noradrenaline utilization are in a direction opposite to that previously found with vasopressin[35].

Structure–activity studies of vasopressin, oxytocin and PLG on other CNS processes show that in some cases PLG has a vasopressin-like effect (memory processes and amnesia), whereas in other situations the effects found with PLG resemble those of oxytocin (heroin self-administration). On morphine tolerance, electrical selfstimulation, rotational behaviour and blood pressure regulation all peptides are active in the same direction (for references see refs. 12, 32, 39, 42). It is noteworthy that in the amygdala a good correlation has been found between the effects of vasopressin and PLG on the reversal of PTZ-induced amnesia and on local dopamine utilization[4,9,37]. Hence, also on amygdaloid dopamine systems PLG appears to exert a vasopressin-like effect.

The results of many studies with PLG obtained so far, point to a central site of action. PLG potentiates DOPA-induced behavioural changes[31], and has influences on morphine tolerance, drug self-administration, amnesia and the central regulation of blood pressure[27]. Also the results of clinical studies with PLG suggest an interaction with the brain[3,13–15]. Originally the peptide was isolated from the brain by Nair et al.[28]. At present, however, it is considered to be very doubtful whether PLG is a naturally occurring peptide. Although PLG is the C-terminal fragment of oxytocin, no PLG could be found after biotransformation of oxytocin with synaptosomal plasma membranes of the rat brain[5]. Using HPLC in combination with a specific radioimmunoassay for PLG Manberg et al.[26] failed to provide evidence for the presence of PLG in the brain. On the other hand, the occurrence of high affinity binding sites for PLG was demonstrated in human and bovine caudate nucleus[8]. In the rat striatum evidence has been presented for a specific PLG receptor functionally coupled to the dopamine-adenylate cyclase system[7].

One possibility to explain the CNS action of PLG is the presence in the brain of a structurally related peptide, Tyr-Pro-Leu-GlyNH$_2$ (Tyr-MIF). Also for this peptide receptors have been found to occur[18]. Unfortunately, no data are available concerning the interaction of Tyr-MIF with central catecholaminergic

systems. The fact that also for oxytocin and vasopressin high affinity binding sites in the rat brain have been found[2,16,30], still leaves the possibility open that PLG exerts its effect via an interaction with the receptors for these neuropeptides.

In conclusion, it can be said that PLG is a peptide with a central site of action. The present results clearly show that in addition to its interaction with brain dopamine neurons, the peptide interacts with distinct noradrenaline containing neurons in the brain. The effects of PLG on dopamine utilization are comparable to those previously found with vasopressin while the effects of PLG on noradrenaline utilization show a striking similarity with those previously obtained with oxytocin. Since biotransformation studies have shown that these two neurohypophyseal hormones are precursor molecules for highly active neuropeptides[5], the influences of these hormones on catecholamine metabolism may be partly mediated by C-terminal fragments generated in the brain.

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REFERENCES

1 Alper, R. H. and Moore, K. E., Systemically administered prolyl-leucyl-glycinamide fails to alter dopaminergic neuronal activity in the rat brain, *J. Pharm. Pharmacol.*, 33 (1981) 259–261.
2 Baskin, D. G., Petracca, F. and Dorsa, D. M., Autoradiographic localization of specific binding sites for [^{3}H]-arginine-vasopressin in the septum of the rat brain with tritium-sensitive film, *Europ. J. Pharmacol.*, 90 (1983) 155–157.
3 Barbeau, A., Roy, M. and Kastin, A. J., Double-blind evaluation of oral L-prolyl-L-leucyl-glycine amide in Parkinson's disease, *Canad. Med. Ass. J.*, 114 (1976) 120–122.
4 Bohus, B., Conti, L., Kovács, G. L. and Versteeg, D. H. G., Modulation of memory processes by neuropeptides: interaction with neurotransmitter systems. In C. Ajmone Marsan and H. Matthies (Eds.), *Neuronal Plasticity and Memory Formation*, Raven Press, New York, 1982, pp. 75–87.
5 Burbach, J. P. H., De Kloet, E. R. and De Wied, D., Oxytocin biotransformation in the rat limbic brain: characterization of peptidase activities and significance in the formation of oxytocin fragments, *Brain Research*, 202 (1980) 401–414.
6 Chiu, S. and Mishra, R. K., Antagonism of morphine-induced catalepsy by L-prolyl-L-leucyl-glycinamide, *Europ. J. Pharmacol.*, 53 (1978/79) 119–125.
7 Chiu, S., Paulose, C. S. and Mishra, R. K., Effect of L-prolyl-L-leucyl-glycinamide (PLG) on neuroleptic-induced catalepsy and dopamine/neuroleptic receptor bindings, *Peptides*, 2 (1981) 105–111.
8 Chiu, S., Wong, Y. W. and Mishra, R. K., Direct evidence for L-prolyl-L-leucyl-glycinamide (PLG) receptor in mammalian brain, *Fed. Proc.*, 64 (1980) abstr. 625.
9 Conti, L., De Wied, D., Versteeg, D. H. G. and Bohus, B., Antiamnesic effect of vasopressin and fragments: structural requirements and site of action, *Proc. 11th Congr. Int. Soc. Psychoneuroendocrinology*, 1980, p. 14.
10 De Wied, D., Behavioural effects of intraventricularly administered vasopressin and vasopressin fragments, *Life Sci.*, 19 (1976) 685–690.
11 De Wildt, D., Verhoef, J. and Witter, A., H-Pro-[^{3}H]Leu-Gly-NH_2: uptake and metabolism in rat brain, *J. Neurochem.*, 38 (1982) 67–74.
12 Dorsa, D. M. and Van Ree, J. M., Modulation of substantia nigra self-stimulation by neuropeptides related to neurohypophyseal hormones, *Brain Research*, 172 (1979) 367–371.
13 Ehrensing, R. H. and Kastin, A. J., Clinical investigations for emotional effects of neuropeptide hormones, *Pharmacol. Biochem. Behav.*, 5, Suppl. 1 (1976) 89–93.
14 Ehrensing, R. H. and Kastin, A. J., Dose-related biphasic effect of prolyl-leucyl-glycinamide (MIF-1) in depression, *Amer. J. Psychiat.*, 135 (1978) 562–566.
15 Ehrensing, R. H., Kastin, A. J., Larsons, P. F. and Bishop, G. A., Melanocyte-stimulating-hormone-release-inhibiting factor-1 and tardive dyskinesia, *Dis. Nerv. Syst.*, 38 (1977) 303–307.
16 Ferrier, B. M., McClorry, S. A. and Cochrane, A. W., Specific binding of [^{3}H]oxytocin in female rat brain, *Canad. J. Physiol. Pharmacol.*, 61 (1983) 989–995.
17 Friedman, E., Friedman, J. and Gershon, S., Dopamine synthesis: stimulation by a hypothalamic factor, *Science*, 182 (1973) 831–832.
18 Kastin, A. J., Lawrence, S. P. and Coy, D. H., Radioimmunoassayable N-Tyr-MIF-I-like activity in rat brain is increased by pinealectomy, *Brain Res. Bull.*, 7 (1981) 697–702.
19 Kastin, A. J., Plotnikoff, N. P., Sandman, C. A., Spirtes, M. A., Kostrzewa, R. M., Paul, S. M., Stratton, L. O., Miller, L. H., Labrie, F., Schally, A. V. and Goldman, H., The effects of MSH and MIF on the brain. In W. E. Stumpf and L. D. Grant (Eds.), *Anatomical Neuroendocrinology*, Karger, Basel, 1975, pp. 290–297.
20 Kastin, A. J., Plotnikoff, N. P., Schally, A. V. and Sandman, C. A., Endocrine and CNS effects of hypothalamic peptides and MSH. In S. Ehrenpreis and I. J. Kopin (Eds.), *Reviews in Neuroscience, Vol. 2*, Raven Press, New York, 1976, pp. 111–148.
21 Kastin, A. J., Schally, A. V. and Kostrzewa, R. M., Possible aminergic mediation of MSH release and of the CNS effects of MSH and MIF-1, *Fed. Proc.*, 39 (1980) 2931–2936.

22 Kostrzewa, R. M., Fukushima, H., Harston, C. T., Perry, K. W., Fuller, R. W. and Kastin, A. J., Striatal dopamine turnover and MIF-1, *Brain Res. Bull.*, 4 (1979) 799–802.
23 Kostrzewa, R. M., Kastin, A. J. and Spirtes, M. A., α-MSH and MIF-1 effects on catecholamine levels and synthesis in various rat brain areas, *Pharmacol. Biochem. Behav.*, 3 (1975) 1017–1023.
24 Kostrzewa, R. M., Spirtes, M. A., Klara, J. W., Christensen, C. W., Kastin, A. J. and Joh, T. H., Effects of L-prolyl-L-leucyl-glycine amide (MIF-1) on dopaminergic neurons, *Pharmacol. Biochem. Behav.*, 5, Suppl. 1 (1976) 125–127.
25 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.
26 Manberg, P. J., Youngblood, W. W. and Kizer, J. S., Development of a radioimmunoassay for Pro-Leu-Gly-NH_2 (PLG or MIF-1): evidence that PLG is not present in rat brain, *Brain Research*, 241 (1982) 279–284.
27 Meisenberg, G. and Simmons, W. H., Centrally mediated effects of neurohypophyseal hormones, *Neurosci. Biobehav. Rev.*, 7 (1983) 263–280.
28 Nair, R. M. G., Kastin, A. J. and Schally, A. V., Isolation and structure of hypothalamic MSH release-inhibiting hormone, *Biochem. Biophys. Res. Commun.*, 43 (1971) 1376–1381.
29 Palkovits, M., *Guide and Map for the Isolated Removal of Individual Cell Groups from Rat Brain*, 1st edn., Akadémiai Kiadó, Budapest, 1980.
30 Pearlmutter, A. F., Costantini, M. G. and Loeser, B., Characterization of [^{3}H]-AVP binding sites in particulate preparations of rat brain, *Peptides*, 4 (1983) 335–341.
31 Plotnikoff, N. P., Minard, F. N. and Kastin, A. J., DOPA potentiation in ablated animals and brain levels of biogenic amines in intact animals after prolyl-leucyl-glycineamide, *Neuroendocrinology*, 14 (1974) 271–279.
32 Schulz, H., Kovács, G. L. and Telegdy, G., Action of posterior pituitary neuropeptides on nigrostriatal dopaminergic system, *Europ. J. Pharmacol.*, 57 (1979) 185–190.
33 Singhal, R. L. and Rastogi, R. B., MIF-1: effects on norepinephrine dopamine and serotonin metabolism in certain discrete brain regions, *Pharmacol. Biochem. Behav.*, 16 (1982) 229–233.
34 Szabó, G., Kovács, G. L., Baláspiri, L. and Telegdy, G., Dose-related effect of the oxytocin fragment prolyl-leucyl-glycinamide on α-MPT-induced catecholamine disappearance and serotonin levels in rat brain, *Neurochem. Int.*, 3 (1981) 411–416.
35 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.
36 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.
37 Van Heuven-Nolsen, D., De Kloet, E. R., De Wied, D. and Versteeg, D. H. G., Microinjection of vasopressin and two related peptides into the amygdala: enhancing effect on local dopamine neurotransmission, *Brain Research*, 293 (1984) 191–195.
38 Van Heuven-Nolsen, D., De Kloet, E. R. and Versteeg, D. H. G., Oxytocin affects noradrenaline utilization in distinct limbic-forebrain regions of the rat brain, *Neuropharmacology*, in press.
39 Van Ree, J. M., Bohus, B., Versteeg, D. H. G. and De Wied, D., Neurohypophyseal principles and memory processes, *Biochem. Pharmacol.*, 27 (1978) 1793–1800.
40 Van Wimersma Greidanus, Tj. B., Van Ree, J. M. and De Wied, D., The role of vasopressin in memory processes, *Pharmacol. Ther.*, 20 (1983) 437–458.
41 Verhoef, J., Veldhuis, H. D. and Witter, A., H-Pro-[^{3}H]Leu-Gly-NH_2: plasma profile and brain uptake following subcutaneous injection in the rat, *J. Neurochem.*, 38 (1982) 1135–1138.
42 Versteeg, C. A. M., Bohus, B. and De Jong, W., Inhibition of centrally-evoked pressor response by neurohypophyseal peptides and their fragments, *Neuropharmacology*, 21 (1982) 1359–1364.
43 Versteeg, D. H. G., Neurohypophyseal hormones and brain neurochemistry, *Pharmacol. Ther.*, 19 (1983) 297–325.
44 Versteeg, D. H. G., Tanaka, M., De Kloet, E. R., Van Ree, J. M. and De Wied, D., Prolyl-leucyl-glycinamide (PLG): regional effects on α-MPT-induced catecholamine disappearance in rat brain, *Brain Research*, 143 (1978) 561–566.
45 Voigt, K. H., Fehm, H. L., Lang, R. E. and Walter, R., The effect of somatostatin and of prolyl-leucyl-glycinamide (MIF) on ACTH release in dispersed pituitary cells, *Life Sci.*, 21 (1977) 739–746.
46 Voith, K., Synthetic MIF analogues. Part II. DOPA potentiation and fluphenazin antagonism, *Arzn. Forsch.*, 27 (1977) 2290–2293.