Microinjection of vasopressin and two related peptides into the amygdala: enhancing effect on local dopamine neurotransmission

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The effects of local microinjection of Arg^8-vasopressin, cyclo[Lys–Gly] and L-Pro–L-Leu–GlyNH$_2$ (PLG) were studied on the α-MPT-induced disappearance of dopamine and noradrenaline in the amygdala and in a number of other brain regions. A dose-dependent increase in dopamine utilization was found in the amygdala after local microinjection of Arg^8-vasopressin, cyclo[Lys–Gly] and PLG at doses of 0.1, 2.7 and 18 pmol respectively. No effects were found on noradrenaline utilization in this brain region. Using a microdissection method, it was found that cyclo[Lys–Gly] enhanced dopamine utilization in the nucl. amygdaloideus centralis, whereas the effect of PLG was mainly located in the nucl. amygdaloideus corticalis. These effects of Arg^8-vasopressin, cyclo[Lys–Gly] and PLG on dopamine utilization in the amygdala are correlated with those on avoidance behavior and can be interpreted as in support of the role of dopamine as neurotransmitter involved in retrieval processes.

In the mammalian brain extensive neuronal networks containing vasopressin and oxytocin have been found to occur, with intra- and extrahypothalamic cell body regions with fibers projecting to the pituitary and various limbic-midbrain and hindbrain regions[4]. Vasopressin and oxytocin are secreted from the pituitary into the bloodstream and act as hormones for peripheral target organs. In the brain vasopressin and oxytocin act as neuropeptides involved in the control of a variety of physiological functions such as brain development, neuroendocrine and cardiovascular regulations, maternal behavior, memory processes, self-stimulation behavior, as well as the development of tolerance and addiction to narcotic drugs (for literature see ref. 16). Whereas for the peripheral action of the hormones the whole molecule appears to be necessary, CNS processes can also be influenced by fragments and derivatives of the molecule[9,11,16]. In particular the structural requirements for the effects on memory processes are well documented[11].

Lesion and microinjection studies have indicated that regions rich in terminals of both neurohypophyseal hormone-containing neurons and catecholamine neurons are the site of action for the effects of vasopressin and oxytocin on memory processes[9,10,20]. Vasopressin and oxytocin and fragments of these neuropeptides affect the activity of distinct catecholamine-containing systems in the brain[20]. A strong correlation was found between the facilitating effect of vasopressin on memory consolidation and its enhancing effect on the utilization of noradrenaline in distinct terminal regions of the coeruleo-telencephalic noradrenaline system[9,10]. It seems likely that not only consolidation but also the retrieval of stored information might be correlated with alterations in the activity of particular catecholamine-containing neurons.

Lesion studies have shown that the amygdala is one of the brain sites involved in memory processes[19]. Results of studies in which vasopressin and related peptides were microinjected into the amygdala showed that these peptides could antagonize disturbed retrieval processes[3], while they did not influence the consolidation process[10]. In the present study we investigated the effect of microinjection of Arg^8-vasopressin, cyclo[Lys–Gly] and L-Pro–L-Leu–GlyNH$_2$ into the amygdala on the α-MPT-induced disappearance of dopamine and noradrenaline in a number of

brain regions including the amygdala. The aim of the present study was to obtain further information as to whether effects in catecholamine metabolism might be related to the behavioral effects of vasopressin and related peptides. The doses used in the present study were therefore in the same range as those used in the behavioral studies (refs. 3 and 10 and L. Conti, D. de Wied, D. H. G. Versteeg and B. Bohus, Proc. 11th Int. Congr. Soc. Psychoneuroendocrinology, Florence, 1980).

Male Wistar rats, weighing 140–160 g at the time of the operation were used. The animals were kept on a controlled light–dark schedule (light on between 06.00 and 20.00 h) and had access to food and tap water ad libitum. Stainless steel canulae (outer diameter 0.6 mm, inner diameter 0.3 mm) were implanted bilaterally into the amygdala under Hypnorm anesthesia. The coordinates were 1.3 mm posterior to bregma, 5.6 mm lateral to the midline and 7.1 mm below the dura at the point of penetration. The canulae were inserted under an angle of 10° to the sagittal plane. A recovery period of 10 days was allowed. The rats were handled during the 5 days preceding the experiment.

Rats were injected intraperitoneally with α-methyl-p-tyrosine methylester HCl (α-MPT; Labkemi AB, Goteborg; 300 mg/kg, i.p.) followed 30 min later by bilateral microinjections of 0.5 μl saline or peptide in 0.5 μl saline. Three hours after local microinjection the rats were decapitated; the brains were rapidly taken out of the skull and frozen on dry ice. The brains were cut in 300 μm sections in a cryostat at —10 °C. From these sections the location of the canulae tips was verified. The amygdala was cut out with

a knife, ultrasonically disintegrated in 400 μl 0.1 N HClO$_4$ and the sonicate was centrifuged (15 min, 15.00 g, 4 °C). Alternatively, brain nuclei were punched out according to the microdissection method described by Palkovits[17], homogenized in 70 μl 0.1 N HClO$_4$ and the homogenates were centrifuged (15 min, 15.000 g, 4 °C). Dopamine and noradrenaline were assayed in a 20 μl aliquot of the supernatant using a radioenzymatic assay[18]. The pellet was redissolved in 1.1 N NaOH and an aliquot of this solution was taken for protein assay[15]. Data are expressed as pg catecholamine/μg protein ± S.E.M. The data were analyzed by one-way ANOVA and subsequently Student's t-test.

Two types of experiments were performed. In the first series of experiments after i.p. injection with α-MPT, rats were microinjected bilaterally into the amygdala with 0.5 μl saline or Arg^8-vasopressin in doses of 0.01, 0.1 and 1 pmol, cyclo[Lys–Gly] in doses of 2.7 or 27 pmol or L-Pro–L-Leu–GlyNH$_2$ in doses of 1.8 or 18 pmol, all in 0.5 μl saline. After decapitation and dissection the amygdala was cut out from 300 μm sections. In a second series of experiments after i.p. injection with α-MPT, rats were microinjected, bilaterally into the amygdala with 0.5 μl saline or cyclo[Lys–Gly] in doses of 0.27, 2.7 or 27 pmol or PLG in doses of 0.18, 1.8 or 18 pmol, all in 0.5 μl saline. After decapitation and dissection the following brain nuclei were punched out: nucl. accumbens, nucl. amygdaloideus basalis, nucl. amygdaloideus centralis, nucl. amygdaloideus corticalis, nucl. amygdaloideus lateralis, nucl. amygdaloideus medialis, nucl. parafascicularis and nucl. paraventricularis.

TABLE I

Effect of microinjection of Arg^8-vasopressin, cyclo [Lys–Gly] and PLG into the amygdala on the α-MPT-induced disappearance of dopamine and noradrenaline in the amygdala

Rats received α-MPT (300 mg/kg, i.p.) 3.5 h and subsequently, saline or peptide (dose indicated in the Table) 3 h prior to decapitation. For further details see text. ANOVA for dopamine $F(7.53) = 2.33$ ($P < 0.05$).

	Dose of the peptide (pmol/0.5 μl saline), microinjected bilaterally							
	Arg^8-vasopressin			cyclo[Lys–Gly]		PLG		
	0	0.01	0.1	1	2.7	27	1.8	18
Dopamine (pg/μg protein)	2.31±0.19	2.63±0.34	1.54±0.23*	1.73±0.34	1.63±0.24*	1.98±0.22	1.90±0.25	1.69±0.14*
Noradrenaline (pg/μg protein)	3.78±0.25	4.21±0.44	3.74±0.46	3.70±0.31	3.81±0.25	4.07±0.28	3.58±0.19	3.74±0.32

* $P < 0.05$ Student's t-test (two-tailed). Mean ± S.E.M. are given, n = 8.

The results of the first series of experiments are shown in Table I. The data represent the concentrations of dopamine and noradrenaline 3.5 h after α-MPT treatment, i.e. 3 h after the saline or peptide injection. A lower concentration of the catecholamine indicates an increased disappearance, whereas a higher concentration represents a decreased disappearance. Bilateral injection of vasopressin, PLG and cyclo[Lys–Gly] into the amygdala increased the disappearance of dopamine in the amygdala at doses of 0.1, 18 and 2.7 pmol, respectively. No effect was found on the α-MPT-induced disappearance of noradrenaline after microinjection of either of the 3 peptides into the amygdala.

Using a microdissection method the effects of PLG and cyclo[Lys–Gly] were further investigated. The results of these experiments are summarized in Table II. At doses of 1.8 and 18 pmol PLG, bilaterally injected into the amygdala, facilitated the disappearance of dopamine in the nucl. amygdaloideus corticalis. At a dose of 2.7 pmol cyclo[Lys–Gly] facilitated the disappearance of dopamine in the nucl. amygdaloideus centralis. In the other nuclei no dopamine could be detected or no significant effects were found (nucl. accumbens). No significant effects were found on noradrenaline disappearance in any of the brain nuclei studied (data not shown).

The present results show that microinjections into the amygdala of Arg[8]-vasopressin as well as of the C-terminal tripeptide of oxytocin, PLG and of the vasopressin derivative cyclo[Lys–Gly] cause a local increase in the utilization of dopamine in this region, whereas they leave the utilization of noradrenaline

unchanged. A further analysis on the site of action of PLG and cyclo[Lys–Gly] within the amygdala revealed that PLG mainly enhanced dopamine utilization in the cortical amygdaloid nucleus, while cyclo[Lys–Gly] did so on the central amygdaloid nucleus. The latter findings could indicate a difference in the site of action of these two peptides, but can also be interpreted as being the result of slight differences in the sites of injection. It should be realized in this respect that the effects of PLG in the central amygdaloid nucleus and those of cyclo[Lys–Gly] in the cortical amygdaloid nucleus were close to significance.

Amnesia is believed to be a deficit in the ability to retrieve stored information. Many treatments, e.g. electroconvulsive shock, CO_2, pentylenetetrazol, α-MPT, diethyldithiocarbamate and protein synthesis inhibitors produce retrograde amnesia[5]. A variety of pharmacological agents, including general stimulants, adrenergic agonists and neuropeptides related to vasopressin, can protect against or reverse amnesia[5]. Most of these compounds also facilitate the retention of the performance in the absence of amnesic agents[5]. Structure–activity studies have shown that not only Arg[8]-vasopressin but also Lys[8]-vasopressin and its C-terminal derivative cyclo[Lys–Gly] and the C-terminal tripeptide of oxytocin, PLG, are effective (ref. 3 and L. Conti, D. de Wied, D. H. G. Versteeg and B. Bohus, Proc. 11th Int. Congr. Soc. Psychoneuroendocrinology, Florence, 1980). Various data indicate that the amygdala is involved in retrieval processes. Evidence has been presented that both catecholamines and vasopressin in the amygdala might have a role in these processes. Local applica-

TABLE II

Effect of microinjection of cyclo[Lys–Gly] and PLG into the amygdala on the α-MPT-induced disappearance of dopamine

Treatment schedule as in Table I; for further details see text. ANOVA for the nucl. amygdaloideus corticalis dopamine (PLG) $F(3,32)$ = 3.42; $P < 0.05$. ANOVA for the nucl. amygdaloideus centralis dopamine (cyclo[Lys–Gly]) $F(3,30)$ = 4.44, $P < 0.02$.

	Dopamine (pg/μg protein) Dose of cyclo[Lys–Gly] pmol/0.5 μl saline microinjected bilaterally				Dopamine (pg/μg protein) Dose of PLG pmol/0.5 μl saline microinjected bilaterally			
	0	0.27	2.7	27	0	0.18	1.8	18
Nucl. accumbens	17.68±1.39	17.26±1.63	18.33±1.99	18.05±2.36	15.66±1.42	16.57±1.28	17.35±0.94	17.21±0.94
Nucl. amygdaloideus centralis	2.69±0.39	1.77±0.20	1.04±0.19*	2.15±0.40	2.20±0.19	2.59±0.53	1.64±0.35	1.98±0.43
Nucl. amygdaloideus corticalis	1.09±0.20	1.37±0.22	0.84±0.16	1.00±0.23	1.65±0.21	1.25±0.36	0.88±0.16*	1.29±0.13*

* $P < 0.05$ Student's t-test (two-tailed). Mean values ± S.E.M. are given, n = 5-8. Dopamine was not detectable in the nucl. amygdaloideus basalis, lateralis, medialis, nucl. parafascicularis and the nucl. paraventricularis.

tion of adrenergic substances in the amygdala has been found to be antiamnesic[6]. Electroconvulsive shock, which impairs the learning abilities of rats, also causes a decrease in the concentration of dopamine in the amygdala[1]. Local microinjection of low doses (in the low pmol range) of Arg[8]-vasopressin, and of cyclo[Lys–Gly] and PLG into the amygdala results in the reversal of pentylenetetrazol-induced amnesia (ref. 3 and L. Conti, D. de Wied, D. H. G. Versteeg and B. Bohus, Proc. 11th Int. Congr. Soc. Psychoneuroendocrinology, Florence, 1980). The present data, showing an enhancing effect on local dopamine utilization after microinjection of these neuropeptides into the amygdala in the same dose range as used in the aforementioned behavioural studies, suggest that vasopressin and related peptides might exert their effects on retrieval processes via dopamine-containing terminals in the amygdala.

A number of neuroanatomical and neurochemical data form indirect evidence in support of this postulate. A relatively high concentration of immunoreactive vasopressin has been measured in the amygdala (for literature see refs. 4 and 16). Vasopressin-and oxytocin-containing neurons are known to terminate in the amygdala, while recently the occurrence of vasopressin-containing cell-bodies in the amygdala has been reported[4]. These systems could well be part of the circuitry of this vasopressin–dopamine interaction. In this respect it is interesting to note that a good correlation exists between dopamine turnover in the amygdala and the performance of rats in operant and passive avoidance behavior[7,8,11,14]. Also, it has been found that the amount of immunoreactive vasopressin in the amygdala is increased immediately after the 24 h retention trial of a passive avoidance response[13].

It is clear that both in the behavioral studies (ref. 3 and L. Conti, D, de Wied, D. H. G. Versteeg and B. Bohus, Proc. 11th Int. Congr. Soc. Psychoneuroendocrinology, Florence, 1980) and in the present neurochemical experiments Arg[8]-vasopressin, cyclo[Lys–Gly] and PLG act in the same way. Autoradiographic techniques enable the characterization and localization of binding sites for vasopressin in the brain[2]. It appears that the central amygdaloid nucleus is one of the limbic brain structures with a high density of such binding sites (Biegon et al., submitted). It is tempting to speculate that the 3 peptides act via these vasopressin binding sites. The dose–response curves are, as previously found in other studies with neuropeptides (for literature see ref. 20), nonlinear, bell-shaped. Arg[8]-vasopressin appears to be much more potent in enhancing dopamine utilization in the amygdala; cyclo[Lys–Gly] and PLG are 27 and 180 times less potent, respectively and, thus, supposedly have a much lower affinity for the putative vasopressin receptor in the amygdala.

In conclusion, the present data suggest that Arg[8]-vasopressin, cyclo[Lys–Gly] and PLG, by interacting with dopamine neurons in the amygdala, might facilitate the retrieval of stored information.

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