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Influence of Z-Prolyl-D-Leucine on α-MPT-Induced Catecholamine Utilization in Specific Mouse Brain Nuclei

GÁBOR L KOVÁCS, LÁSZLÓ ACSAI, ÁGNES TIHANYI, MÁRIA FALUDI AND GYULA TELEGDY

Institute of Pathophysiology, University Medical School, Szeged, Hungary

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KOVACS, G L, L ACSAI, A TIHANYI, M FALUDI AND G TELEGDY *Influence of Z-Prolyl-D-Leucine on α-MPT-induced catecholamine utilization in specific mouse brain nuclei* PHARMACOL BIOCHEM BEHAV **18**(3) 345–349, 1983 —The synethetic dipeptide Z-Prolyl-D-Leucine (Z-Pro-D-Leu) inhibits the development of tolerance to and dependence on morphine in mice The possible mode of action of the dipeptide was studied by measuring the α-MPT-induced disappearance of noradrenaline (NA) and dopamine (DA) either by a spectrofluorimetric assay in major brain areas (lower brainstem, striatum) or by a radioenzymatic assay in specific brain nuclei For the latter purpose, mouse brain nuclei containing mainly the cell body areas (nucl tractus solitarii, locus coeruleus, substantia nigra, area tegmentalis ventralis) or some selected terminal projections (nucl caudatus nucl accumbens, gyrus dentatus hippocampi, nucl raphe dorsalis) of major NA- and DA-containing pathways were selected In the lower brainstem studied as a whole, the dipeptide did not affect the utilization of either NA or DA Analysis of the data on the NA utilization in specific brain nuclei however, revealed that the dipeptide affected NA disappearance in some mesencephalic-limbic nuclei which receive noradrenergic innervation from the dorsal noradrenergic bundle (e g , nucl raphe dorsalis, area tegmentalis ventralis, gyrus dentatus) NA utilization in the cell body region of the same pathway (locus coeruleus), however, was not affected by the dipeptide The dipeptide facilitated DA utilization in the main terminal area of the mesolimbic DA-ergic projection (nucl accumbens), whereas the same treatment inhibited DA utilization in the main terminal region of the nigro-striatal DA-ergic pathway (nucl caudatus) The data suggest that localized changes in NA and DA utilization following Z-Pro-D-Leu might be important for peptide-induced changes in morphine tolerance and dependence

Z-Prolyl-D-Leucine Noradrenaline Dopamine Specific nuclei

NEUROHYPOPHYSEAL hormones affect tolerance to and dependence on morphine and heroin [12,24] More recently, it has been described that di- and tripeptides derived from these hormones inhibit the development of a physical dependence on morphine in mice [10, 18, 25, 26] One of the most active sequences in this respect is Z-Pro-D-Leu, a synthetic dipeptide which contains the D enantiomer of leucine [10,18]

Relatively little is known about the mode of action of Z-Pro-D-Leu on morphine tolerance and dependence It has been shown by Kovács *et al* [10,11] that approximately 1/100 times lower amounts of the dipeptide are required to affect naloxone-precipitated morphine withdrawal after intracerebroventricular administration than following a peripheral challenge with the peptide This finding suggests that the dipeptide is capable of influencing the central nervous system directly in order to affect morphine dependence As far as the mode of action is concerned within the central nervous system, the dipeptide did not affect the antinociceptive effect of acute morphine treatment [10] in morphine-naive mice, indicating that an interaction of Z-Pro-D-Leu with endogenous opioid binding sites is not likely As an alternative hypothesis, it was suggested that the dipeptide affects CNS mechanisms located functionally distal from opiate receptors Accordingly, it was shown [10,11] that acute administration of Z-Pro-D-Leu to morphine-naive mice decreased the steady-state level of NA in the lower brainstem, while a subchronic treatment with the dipeptide resulted in an additional decrease in the steady-state level of brainstem DA as well The fact that Z-Pro-D-Leu affected the steady-state levels of NA or DA raised the possibility that the utilization of these catecholamines could also be changed by the dipeptide The involvement of noradrenergic and dopaminergic neurotransmission in the development of morphin tolerance and dependence (for summary see [2,20]) favors the idea that an altered catecholamine utilization due to peptide treatment may correlate with peptide-induced changes in morphin tolerance and dependence

The present study was undertaken to investigate the influence of Z-Pro-D-Leu on α-MPT-induced catecholamine disappearance in major brain regions and to test the postulate that the dipeptide acts on particular monoamine systems in the brain Therefore, the utilization of NA and DA was measured in mouse brain nuclei either containing the cell body areas of major noradrenergic (nucl tractus solitarii, locus coeruleus) and dopaminergic (substantia nigra, area tegmentalis ventralis) pathways, or which received axon terminals of the same projections (e g , nucl caudatus, nucl

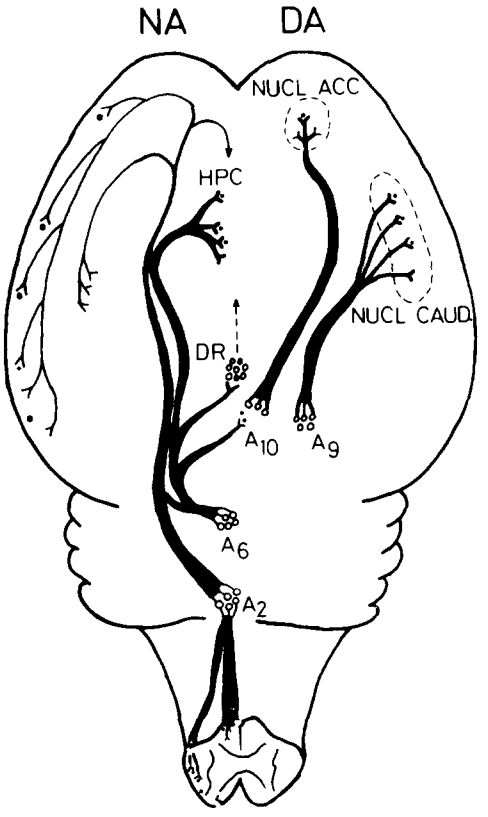

FIG 1 Schematic representation of mouse brain nuclei in which the utilization or noradrenaline or dopamine was studied Abbreviations NA=noradrenaline pathways DA=dopamine pathways A_2=nucleus tractus solitarii, A_6=locus coeruleus DR=nucleus raphe dorsalis, HPC=gyrus dentatus hippocampi, A_{10}=area tegmentalis ventralis, A_9=substantia nigra, NUCL ACC =nucleus accumbens, NUCL CAUL =nucleus caudatus

accumbens, nucl raphe dorsalis, gyrus dentatus hippocampi) (for review see [5, 6, 13, 22]) (Fig 1)

METHOD

Animals

Male CFLP mice, 18–22 g body weight, were used The animals were maintained on a standard illumination schedule of 12 hr (light on between 6 a m and 6 p m) with food and water available ad lib Mice were housed 10 per cage

Biochemical Methods

An intraperitoneal injection of α-methyl-p-tyrosine methyl ester HCl (α-MPT, Sigma, 250 mg/kg) was followed, 30 min later, by subcutaneous administration of Z-Prolyl-D-Leucine (Peninsula Laboratories, 50 μg/animal) or vehicle (6% ethanol dissolved in physiological saline (v/v) 0 1 ml/10 g b w) Three hours after the subcutaneous treatment, i e , 3 5 hr after the administration of the tyrosine hydroxylase inhibitor α-MPT, the mice were killed by decapitation The brains were rapidly taken out of the skull and frozen on dry ice Subsequently, the brains were stored on dry ice until processing

Spectrofluorimetric estimation of catecholamine utilization in major brain regions The lower brainstem (pons+medulla) and the striatum were dissected and the NA and DA levels were estimated with the spectrofluorimetric assay of Jacobowitz and Richardson [7] as modified by Szabo *et al* [21] Transmitter levels were expressed as nmol transmitter/g wet tissue

Radioenzymatic estimation of catecholamine utilization in specific mouse brain nuclei The brains were cut into 300 μm sections in a cryostat at -10°C Brain nuclei were dissected under a stereomicroscope with hollow needles according to the method of Palkovits [16] originally described for rat brain nuclei For localization of mouse brainstem and mesencephalic nuclei, the stereotaxic map of Kovac and Denk [9] was used Forebrain nuclei were punched according to the principles of Palkovits [7] described for rat brain nuclei

(a) Nucl accumbens was removed from the forebrain slice in which the anterior commissure the diagonal band and two separate optic nerves appeared in the same section (For rat brain coordinates the 0 (bregma) point is indicated by the appearance of two intact fornicis and one single horizontal anterior commissure in the midline Taking the same appearance of these structures in the mouse brain as the 0 point, the nucl accumbens was removed from the section located 600–900 μm frontally to the 0 point) The dorsomedial portion of the nucl accumbens was removed from both sides

(b) Samples from the nucl caudatus were taken bilaterally from the above described section as well as from the next slice located 300 μm caudally The dorsolateral part of the nucl caudatus was punched, i e , the area located in the vicinity (ventral to) to corpus callosum

The tissue pellets were homogenized in 50 μl 0 1 N $HClO_4$ A 20 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, 6000 g, +4°C) NA and DA were assayed in 20 μl samples of the supernatant, according to the radioenzymatic microassay of Van der Gugten *et al* [23], using liver catechol-O-methyltransferase and S-adenosyl-methyl [^{3}H]-methionine (Amersham, England) The two catecholamines were separated from each other by thin-layer chromatography [15] Data were calculated as fmol catecholamine/μg protein ±S E M

Statistical Analysis

The data were analyzed by one-way ANOVA and subsequently by Student's t-test (two-tailed) A p-value of 0 05 was considered to indicate statistical significance

RESULTS

Data on catecholamine utilization in major brain regions are summarized in Table 1 Injection of α-MPT resulted in a significant decrease in the NA and DA contents of both the lower brainstem and the striatum Compared to α-MPT-treated controls, Z-Pro-D-Leu did not influence the NA or DA disappearance in the brainstem, while the dipeptide significantly prevented the α-MPT-induced decrease in the DA content of the striatum (Table 1)

A more complex pattern of changes was revealed by analysing the catecholamine utilization in specific brain nuclei Inhibition of NA synthesis by α-MPT caused a highly significant decrease in the NA content of each brain nucleus studied Compared to the α-MPT-injected controls, Z-

TABLE 1

EFFECTS OF Z-PROLYL-D-LEUCINE ON α-MPT-INDUCED NORADRENALINE AND DOPAMINE DISAPPEARANCE IN MAJOR BRAIN REGIONS

	Noradrenaline	Significance	Dopamine	Significance
Brainstem				
1 Vehicle + Vehicle	3 66 ± 0 36* (6)	—	7 19 ± 0 50 (6)	—
2 α-MPT + Vehicle	1 83 ± 0 18 (9)	0 001 vs 1	4 18 ± 0 92 (9)	0 05 vs 1
3 α-MPT + Z-Pro-D-Leu	1 72 ± 0 12 (9)	NS vs 2	4 84 ± 0 92 (10)	NS vs 2
Striatum				
1 Vehicle + Vehicle	n d		48 63 ± 3 20 (6)	—
2 α-MPT + Vehicle	n d		27 25 ± 1 11 (10)	0 001 vs 1
3 α-MPT + Z-Pro-D-Leu	n d		33 14 ± 2 16 (9)	0 05 vs 2

*Mean ± S E M expressed in nmol/g tissue
( ) Number of animals

TABLE 2

EFFECT OF Z-PROLYL-D-LEUCINE ON α-MPT-INDUCED DISAPPEARANCE OF NORADRENALINE IN BRAIN NUCLEI

Brain Nuclei	Intact Control	α-MPT+Vehicle	α-MPT+Z-Pro-D-Leu	Disappearance	Significance
Nucl tractus solitarii	101 89 ± 18 20* (7)	52 71 ± 10 16 (14)	63 53 ± 10 87 (13)	88%	NS
Locus coeruleus	318 72 ± 38 71 (7)	201 83 ± 23 88 (14)	203 18 ± 42 37 (14)	99%	NS
Nucl raphe dorsalis	51 42 ± 10 40 (6)	25 47 ± 4 08 (13)	39 60 ± 3 90 (15)	64%	0 05
Substantia nigra	43 08 ± 9 04 (7)	12 71 ± 1 83 (14)	16 31 ± 2 48 (16)	77%	NS
Area tegmentalis ventr	51 12 ± 11 17 (7)	26 71 ± 2 65 (13)	17 55 ± 2 24 (16)	152%	0 02
Gyrus dentatus hippoc	29 25 ± 4 78 (7)	13 53 ± 1 53 (11)	8 98 ± 0 94 (15)	151%	0 02
Nucl caudatus	n d	n d	n d		
Nucl accumbens	n d	n d	n d		

*Mean ± S E M expressed in fmol transmitter/μg protein
α-MPT treatment resulted in a significant ($p<0$ 001) decrease in the noradrenaline level in all brain regions studied
Disappearance of catecholamines in the α-MPT+Vehicle group was taken as 100% and the data are expressed as a percentage of this control group According to this calculation, disappearance values higher than 100% indicate an accelerated catecholamine utilization
Significance Significant differences between groups treated with α-MPT+Vehicle and α-MPT+Z-Pro-D-Leu
n d Monoamine not detectable
( ) Number of animals

Pro-D-Leu prevented the decrease of NA in the nucl raphe dorsalis and facilitated the α-MPT-induced decrease of NA in the area tegmentalis ventralis and gyrus dentatus of the dorsal hippocampus The dipeptide was without significant effects on the disappearance of NA in any of the other brain nuclei studied (Table 2)

Endogenous levels of DA were significantly decreased in all brain regions by administration of α-MPT Injection of Z-Pro-D-Leu inhibited the α-MPT-induced decline in the DA content of the nucl caudatus In the nucl accumbens, on the other hand, the dipeptide facilitated the disappearance of DA The DA disappearance in the brainstem (nucl tractus solitarii, locus coeruleus) or mesencephalic (nucl raphe dorsalis, substantia nigra, area tegemntalis ventralis) nuclei was not affected by the dipeptide (Table 3)

DISCUSSION

Previous studies have shown that the synthetic dipeptide Z-Pro-D-Leu inhibits the development of tolerance to and dependence on morphine in mice [10, 11, 18] More recent data raised the possibility that the effect of the dipeptide on morphine tolerance and dependence might be related to peptide-induced changes in brain monoaminergic neurotransmission [10] In support of this notion, it has been found that acute administration of Z-Pro-D-Leu decreased the steady-state level of NA in the lower brainstem (pons+medulla), but it has no effect on the DA in the striatum The present experiment was designed to analyse the α-MPT-induced disappearance of NA and DA following Z-Pro-D-Leu treatment Since α-MPT is known to inhibit the synthesis of both catecholamines at the level of tyrosine hydroxylase, changes observed due to peptide treatment administered after α-MPT treatment reflect changes in the utilization (release, reuptake and metabolism) of the catecholamines In the present experiment the dipeptide did not affect the utilization of either NA or DA in the lower brainstem, but decreased DA utilization in the striatum Since the dipeptide decreased the steady-state level of brainstem NA [10], but did not affect the utilization of this

TABLE 3

EFFECT OF Z-PROLYL-D-LEUCINE ON α-MPT-INDUCED DISAPPEARANCE OF DOPAMINE IN BRAIN NUCLEI

Brain Nuclei	Intact Control	α-MPT+Vehicle	α-MPT+Z-Pro-D-Leu	Disappearance	Significance
Nucl tractus solitarii	20 05 ± 4 38† (6)	11 10 ± 1 31 (10)	7 97 ± 1 18 (13)	140%	NS
Locus coeruleus	16 52 ± 3 40 (7)	8 29 ± 1 44 (12)	5 09 ± 1 24 (12)	163%	NS
Nucl raphe dorsalis	18 41 ± 1 96 (6)	7 64 ± 1 31 (13)	6 86 ± 1 24 (12)	111%	NS
Substantia nigra	54 39 ± 12 67 (7)	16 72 ± 3 33 (16)	14 76 ± 1 96 (16)	113%	NS
Area tegmentalis ventr	40 94 ± 11 49 (6)	17 83 ± 2 55 (14)	17 50 ± 2 94 (16)	102%	NS
Gyrus dentatus hippoc	n d	n d	n d	—	—
Nucl caudatus	420 14 ± 79 47 (6)	237 56 ± 24 94 (13)	334 53 ± 24 88 (14)	71%	0 05
Nucl accumbens	397 48 ± 69 74 (6)	242 33 ± 17 96 (16)	130 73 ± 22 14 (15)	160%	0 05

For notes see Table 2

monoamine in the same structure (present study), one might assume that the decrease in the steady-state level was brought about by inhibition of NA synthesis induced by the dipeptide Since the dipeptide decreased DA utilization in the striatum after inhibition of DA synthesis by α-MPT, but this decreased utilization was not reflected in changes of the steady-state level in normal animals not treated with α-MPT, [10] it might be assumed that the dipeptide exerted a dual effect on the DA metabolism in the striatum In addition to the decrease in the utilization of the monoamine, the dipeptide might have decreased the synthesis of DA as well

A more detailed analysis of NA and DA utilization was performed in specific mouse brain nuclei with a radioenzymatic microassay As in the lower brainstem as a whole, Z-Pro-D-Leu did not affect the utilization of NA in the two brainstem nuclei which contain noradrenergic cell bodies (nucl tractus solitarii and locus coeruleus) and give rise to the dorsal and ventral noradrenergic bundle [5 7] The dorsal noradrenergic bundle is known to project from the locus coeruleus to various cortical, limbic and mesencephalic structures [6] In some of these terminal projections of the dorsal noradrenergic bundle the dipeptide affected the utilization of NA Thus, the dipeptide inhibited NA utilization in the dorsal raphe nucleus, a brain site which contains serotonin cell bodies around which NA terminals of the dorsal noradrenergic bundle impinge [1,19] Conversely, facilitation of NA utilization was found in the area tegmentalis ventralis, a brain site which contains DA cell bodies projecting to limbic structures (=mesolimbic DA projection [4, 5, 6, 13]) In the gyrus dentatus of the dorsal hippocampus, which is also innervated by the dorsal noradrenergic bundle [3,8], the dipeptide likewise facilitated NA disappearance The reason why the dipeptide exerts inhibitory as well as facilitatory effects on the NA utilization in different brain areas innervated by the same noradrenergic pathway remains to be elucidated It could be that the dipeptide does not influence NA-ergic neurotransmission directly, but via other transmitter systems, which in turn affect NA-ergic neurotransmission differentially

Similarly to NA utilization, differential effects were observed in different DA-ergic pathways following Z-Pro-D-Leu The dipeptide slightly (but not significantly) accelerated DA utilization in the two mainly NA-containing brainstem nuclei (nucl tractus solitarii and locus coeruleus) Since NA utilization was not affected in these loci, it is not likely that an increased precursor synthesis of NA occurred There was no effect of Z-Pro-D-Leu on the DA utilization in the mesencephalic nuclei which contain DA cell bodies of the mesolimbic (area tegmentalis ventralis) and nigro-striatal (substantia nigra) DA-ergic projections In the most prominent terminal areas of these DA-ergic pathways, however marked changes were observed following Z-Pro-D-Leu In the nucl accumbens—a major terminal region of the mesolimbic DA-ergic projection—the dipeptide facilitated utilization of DA In the nucl caudatus, on the other hand, inhibition of DA utilization was found following Z-Pro-D-Leu (the same effect as was found in the whole striatum by spectrofluorimetric analysis) It might be supposed that either the mesolimbic and nigro-striatal DA-ergic projections respond to Z-Pro-D-Leu differentially, or the effect of the dipeptide on DA utilization is mediated by other neurotransmitter systems which exert opposite effects on the mesolimbic versus nigro-striatal DA terminals

In conclusion, the data support the idea that Z-Pro-D-Leu exerts localized changes in the utilization of NA in limbic-mesencephalic terminal areas of the dorsal noradrenergic bundle and in the DA utilization of the main terminal projections of the mesolimbic and nigro-striatal DA-ergic pathways Which of these effects is causally related to the influence of Z-Pro-D-Leu on morphine tolerance and dependence remains to be investigated

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