Pharmacology Biochemistry & Behavior, Vol. 21, pp. 345–348, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Drugs Affecting Brain Dopamine Interfere With the Effect of Z-Prolyl-D-Leucine on Morphine Withdrawal

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Received 29 November 1983

KOVÁCS, G. L., G. TELEGDY AND K. HÓDI. *Drugs affecting brain dopamine interfere with the effect of Z-prolyl-D-leucine on morphine withdrawal.* PHARMACOL BIOCHEM BEHAV 21(3) 345–348, 1984.—The dipeptide Z-prolyl-D-leucine (Z-Pro-D-Leu) has been demonstrated to inhibit the development of tolerance to and dependence on morphine in the mouse. Since the dipeptide affects dopamine (DA) utilization in the terminal regions of the mesolimbic and nigrostriatal DA-ergic projections, the question has been studied of whether DA-ergic mechanisms are involved in the action of Z-Pro-D-Leu on morphine withdrawal. Both inhibition of tyrosine hydroxylase by α-methyl-p-tyrosine (α-MPT) and inhibition of DA receptors by pimozide interfere with the effect of Z-Pro-D-Leu on naloxone-precipitated morphine withdrawal. Inhibition of serotonin (5-HT) synthesis by DL-p-chlorophenylalanine (PCPA), on the other hand, does not modify the effect of the dipeptide. The results argue for a role of DA-ergic mechanisms in the effect of Z-Pro-D-Leu on the development of morphine dependence.

Z-Pro-D-Leu Morphine withdrawal DA synthesis Receptors

THE synthetic dipeptide Z-Pro-D-Leu, which is a derivative of the C-terminal part of oxytocin, has been shown to inhibit the development of tolerance to and dependence on morphine in the mouse [3, 4, 8, 10]. Since the dipeptide did not affect the analgesic effect of acute morphine treatment in morphine-naive mice [3], the interaction of Z-Pro-D-Leu with endogenous opioid binding sites is not likely. As an alternative mode of action, Kovács *et al.* [3,5] studied the influence of Z-Pro-D-Leu on the activity of various neurotransmitter pathways in the mouse brain. It was found that a dose of Z-Pro-D-Leu which attenuates morphine withdrawal also increases the utilization of DA in the nucleus accumbens [5], a brain site which contains mesolimbic DA terminals originating from the ventral tegmental area [2,7]. In the nucleus accumbens, a terminal region of the nigrostriatal DA-ergic pathway [2], on the other hand, Z-Pro-D-Leu inhibited the utilization of DA. Since the development of morphine tolerance/dependence and also morphine withdrawal is accompanied by characteristic changes in the utilization of DA in these brain regions [6], the data raise the possibility that peptide-induced changes in the activity of the mesolimbic and/or nigrostriatal DA-ergic projections might be causally related to the ability of the dipeptide to attenuate morphine withdrawal. This possibility has been tested in the present experiment by investigating the effect of Z-Pro-D-Leu on morphine withdrawal in normal mice, as compared to animals in which catecholamine synthesis or DA receptors were blocked by drugs. However, since Z-Pro-D-Leu has also been shown to decrease the steady-state level of 5-HT in the lower brainstem [3], the involvement of 5-HT-ergic mechanisms was investigated as well.

METHOD

Animals

Male CFLP mice, 25–30 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.). Food and drinking water were available ad lib. Mice were housed 10 per cage.

Naloxone-Precipitated Withdrawal Syndrome

The naloxone-precipitated morphine withdrawal syndrome was tested according to previously published methods [3,11]. Briefly, mice were anesthetized with ether vapour and a morphine pellet was implanted under the skin on the neck. The pellet contained 37.5 mg morphine-HCl (for the exact composition of the pellet, see [3]). Three days after pellet implantation, naloxone (1 mg/kg, Endo, Du Pont de Nemours GmbH) was injected subcutaneously and the precipitated abstinence syndrome was measured via the latency of onset of stereotyped jumpings.

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TABLE 1

INTERACTION OF VARIOUS DRUGS AND Z-PRO-D-LEU ON MORPHINE WITHDRAWAL

Groups	−24 hr	−4 hr	−2 hr	0 hr	+72 hr	Measured Parameter
Control	Vehicle	Vehicle	Vehicle	Morphine	Naloxone	Withdrawal
	Vehicle	Vehicle	Decapit.	—	—	DA, 5-HT
	Vehicle	Vehicle	—	—	Decapit.	DA, 5-HT
Z-Pro-D-Leu	Vehicle	Vehicle	Peptide	Morphine	Naloxone	Withdrawal
α-MPT	Vehicle	α-MPT	Vehicle	Morphine	Naloxone	Withdrawal
	Vehicle	α-MPT	Decapit.	—	—	DA, 5-HT
	Vehicle	α-MPT	—	—	—	DA, 5-HT
α-MPT + Z-Pro-D-Leu	Vehicle	α-MPT	Peptide	Morphine	Naloxone	Withdrawal
Pimozide	Vehicle	Pimozide	Vehicle	Morphine	Naloxone	Withdrawal
Pimozide + Z-Pro-D-Leu	Vehicle	Pimozide	Peptide	Morphine	Naloxone	Withdrawal
PCPA	PCPA	Vehicle	Vehicle	Morphine	Naloxone	Withdrawal
	PCPA	Vehicle	Decapit.	—	—	DA, 5-HT
	PCPA	Vehicle	—	—	Decapit.	DA, 5-HT
PCPA + Z-Pro-D-Leu	PCPA	Vehicle	Peptide	Morphine	Naloxone	Withdrawal

Estimation of Brain Monoamines (DA and 5-HT)

Mice were decapitated and the brains were quickly removed. The lower brainstem (pons+medulla) and the striatum were assayed for DA and 5-HT via spectrofluorimetric method of Szabó *et al.* [9]. Biochemical data are expressed in nmol transmitter/g tissue.

Treatments

Z-Prolyl-D-leucine (Peninsula) was dissolved in physiological saline containing 6% ethanol. A single dose of 50 μg/animal was injected 2 hr prior to the implantation of the morphine pellet. DL-p-chlorophenylalanine (PCPA, Sigma) or α-methyl-p-tyrosine (α-MPT, Sigma) was injected IP 24 or 2 hr prior to the peptide injection, respectively. Pimozide (0.25 mg/kg) was injected 2 hr prior to the peptide injection. Control mice received the equivalent amounts of vehicle. A scheme of treatments and testings is depicted in Table 1.

Statistical Analysis

Data were analyzed with ANOVA, followed by Scheffe's test. Data on latencies were first transformed to reciprocal scores. A probability level of 0.05 was accepted as indicating significant differences.

RESULTS

Biochemical data on brain DA and 5-HT are summarized in Table 2. Animals were decapitated either at the time when peptide treatment should have been given or at the time when morphine withdrawal should have been tested. At the time of peptide challenge, decreased DA levels were measured in both the striatum and the lower brainstem of the mice treated with α-MPT. The 5-HT level of the same animals, on the other hand, remained unaffected following α-MPT treatment. DA levels returned to normal later on, and thus at the time of morphine withdrawal the DA levels were in the control range. Similar effects were found on 5-HT levels following PCPA treatment: 5-HT was significantly lower at the time of peptide challenge, but had returned to normal by the time of morphine withdrawal.

Behavioral data are summarized in Table 3. Z-Pro-D-Leu significantly delayed the latency of onset of withdrawal jumpings. This effects was absent in the animals in which catecholamine synthesis was inhibited by α-MPT. The synthesis inhibitor (α-MPT) alone, however, did not influence morphine withdrawal. Inhibition of 5-HT synthesis by PCPA neither modified the effect of Z-Pro-D-Leu, nor affected morphine withdrawal itself. The DA receptor antagonist pimozide exerted the same effect as that of the catecholamine synthesis inhibitor; thus, it prevented the effect of Z-Pro-D-Leu on morphine withdrawal, but did not influence the withdrawal reaction in itself.

DISCUSSION

The present data confirm earlier findings that the effect of Z-Pro-D-Leu, a synthetic dipeptide derived from the C-terminal part of oxytocin, attenuates the development of tolerance to and dependence on morphine, and suggest that this effect is due in part to an interaction of the dipeptide with DA-ergic mechanisms in the mouse brain. Accordingly, it has been found [5] that shortly after peripheral treatment Z-Pro-D-Leu facilitates DA utilization in the nucleus accumbens, a major terminal region of the mesolimbic DA-ergic projection [2]. However, whether this increased DA turnover was related causally or downstream to (and thus not involved in) the effect of the dipeptide was not answered by

TABLE 2

EFFECTS OF α-MPT AND PCPA ON DOPAMINE AND SEROTONIN LEVELS IN THE MOUSE BRAIN

Brain Regions/ Monoamine	Control		α-MPT Treatment[a]		PCPA Treatment[a]	
	Time of Peptide Challenge	Time of Withdrawal	Time of Peptide Challenge	Time of Withdrawal	Time of Peptide Challenge	Time of Withdrawal
Striatum						
DA:	39.7 ± 2.0[c]	41.1 ± 2.3*	29.2 ± 3.1†	37.1 ± 4.4*	37.4 ± 5.0*	40.8 ± 3.2*
5-HT:	5.9 ± 0.3	6.0 ± 0.4*	5.7 ± 0.3*	6.4 ± 0.5*	3.9 ± 0.2†	6.2 ± 0.5*
Lower Brainstem						
DA:	6.9 ± 0.3	7.0 ± 0.4*	4.4 ± 0.2†	8.0 ± 0.6*	6.3 ± 0.5*	6.4 ± 0.6*
5-HT:	5.4 ± 0.2	5.3 ± 0.4*	5.0 ± 0.5*	5.2 ± 0.6*	3.1 ± 0.2‡	4.9 ± 0.2*

[a]200 mg/kg α-MPT (IP) was injected 4 hr prior to the implantation of the morphine pellet, i.e., 2 hr prior to the injection of Z-Pro-D-Leu. Withdrawal symptoms were tested 72 hr after the morphine pellet was implanted. In conclusion, mice were decapitated either 2 or 76 hr after a single injection of α-MPT.

[b]200 mg/kg PCPA (IP) was injected 24 hr prior to the injection of Z-Pro-D-Leu, and thus mice were decapitated for 5-HT assay either 24 or 98 hr after a single injection of PCPA.

[c]Mean ± SEM of 8 mice, expressed in nmol transmitter/g wet tissue.

*Not significant.

†$p<0.01$.

‡$p<0.001$.

TABLE 3

INFLUENCE OF DRUGS ON PEPTIDE-INDUCED ATTENUATION OF WITHDRAWAL SYNDROME IN MICE

Treatments	No.	Latency of Withdrawal*	Significance†
1. Saline + 6% Ethanol	(40)	145 ± 9	
2. Saline + Z-Pro-D-Leu	(33)	186 ± 14	0.001 vs. 1
3. α-MPT + 6% Ethanol	(17)	158 ± 11	NS vs. 1
4. α-MPT + Z-Pro-D-Leu	(16)	162 ± 17	NS vs. 3
5. PCPA + 6% Ethanol	(21)	159 ± 16	NS vs. 1
6. PCPA + Z-Pro-D-Leu	(16)	199 ± 21	0.05 vs. 5
7. PIMO + 6% Ethanol	(18)	166 ± 15	NS vs. 1
8. PIMO + Z-Pro-D-Leu	(9)	151 ± 21	NS vs. 7

*Mean ± SEM in sec.

α-MPT=200 mg/kg α-MPT treatment (IP) injected 2 hr prior to the peptide or 6% ethanol administration.

PCPA=200 mg/kg PCPA treatment (IP) injected 24 hr prior to the peptide or 6% ethanol administration.

PIMO=0.25 mg/kg pimozide (IP) injected 2 hr prior to the peptide or 6% ethanol treatment.

†Analysis of variance.

NS=not significant.

the biochemical experiments. The present studies were undertaken to resolve this problem.

The major methodological problem relating to this question is that the development of morphine dependence and the expression of withdrawal symptoms are separate phenomena but both can be modified in the same direction by a particular treatment. Since the latency (or the severity) of withdrawal symptoms is generally used to measure both phenomena, separation—although essential—might be rather difficult. In this experiment the dipeptide was injected as a single treatment exclusively prior to the implantation of the morphine pellet, and hence the delay in the onset of withdrawal jumpings 3 days later argues for an effect of the dipeptide on the development of morphine dependence. It is not likely that intact Z-Pro-D-Leu is present 76 hr after a single injection and can directly influence the expression of withdrawal symptoms. Monoamine synthesis and receptor inhibitors were given as a single treatment prior to the injection with the dipeptide. Injection times were selected so that the drug effects should be clearly present (maximal) at the time of peptide treatment, but should be absent at the time when morphine withdrawal was precipitated by naloxone (when the expression of withdrawal symptoms could be modified by drugs). As a result of this treatment schedule, neither the

synthesis inhibitors (α-MPT, PCPA), nor the DA receptor antagonist affected the latency of onset of withdrawal from morphine. This lack of effects does not contradict the great number of literature data pointing to the role of brain catecholamines and 5-HT in the expression of morphine withdrawal (for review, see [1,12]).

As a major conclusion of this paper, it has been found that partial inhibition of tyrosine hydroxylase by α-MPT completely prevented the effect of Z-Pro-D-Leu on the development of morphine dependence. It should be noted that a single injection of 200 mg/kg α-MPT resulted in a 23–35% decrease of the striatal and brainstem DA (and also of NA, not documented here) contents at the time of peptide challenge. Since pretreatment with pimozide, a DA receptor antagonist, resulted in identical blockade of the effect of the dipeptide, it is tempting to conclude that intact DA-ergic innervation is essential for Z-Pro-D-Leu to affect the development of morphine dependence.

It is of interest to note that subchronic, but not acute Z-Pro-D-Leu treatment decreased the steady-state level of 5-HT in the mouse brainstem [3]. Since partial inhibition of 5-HT synthesis, resulting in a 40–50% decrease of the striatal and brainstem 5-HT, did not modify the effect of Z-Pro-D-Leu, it is likely that 5-HT-ergic mechanisms play little if any role in mediating the action of Z-Pro-D-Leu on the development of morphine dependence. It can not be excluded, however, that some other aspects of morphine withdrawal which were not studied here (e.g., temperature regulation, blood pressure, etc.) are affected by brain 5-HT.

Taken together, the data indicate that inhibition of DA synthesis or receptors interferes with the effect of Z-Pro-D-Leu on the development of morphine withdrawal, and therefore suggest that altered DA metabolism [5] might be causally related to this effect.

ACKNOWLEDGEMENTS

Naloxone was kindly provided by Endo Laboratories (Endo Du Pont de Nemours GmbH). The technical assistance of Mrs. Katalin Kovács is acknowledged.

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