Pharmacology Biochemistry & Behavior, Vol. 35, pp. 47–50. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Intensification and Attenuation of Morphine Dependence by D-Aspartic Acid and PLG

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Received 17 April 1989

KOYUNCUOĞLU, H., M. GÜNGÖR, H. SAĞDUYU AND F. ARICIOĞLU. *Intensification and attenuation of morphine dependence by D-aspartic acid and PLG.* PHARMACOL BIOCHEM BEHAV **35**(1) 47–50, 1990.—The inhibition by opiates and the sudden normalization by opioid antagonists of the brain L-asparaginase activity (BAA) have previously been reported to be the main factors in the development of physical dependence and the manifestation of precipitated abstinence syndrome, respectively. As a result, L-asparaginase inhibitors D-aspartic acid and prolyl-leucyl-glycinamide (PLG) were separately given to mice and rats either just after morphine (M)-containing pellet implantation or 15 min before naloxone (NL)-precipitated abstinence syndrome. The animals treated in this manner were used to assess the intensity of the physical dependence and to determine the BAA. D-ASP or PLG administration following pellet implantation significantly increased all of the observed signs such as flying, jumping, wet dog shake and writhing. When D-ASP or PLG were given 15 min before precipitated abstinence they significantly decreased the number of the signs. The determination of the BAA showed significant decreases or increases more or less parallel to the severity of the physical dependence on M. The intensification of physical dependence by D-ASP or PLG given just after the pellet implantation was attributed to their additional inhibitory effect to that of M on the BAA at the beginning of the physical dependence development. The attenuating effect of BAA inhibitors D-ASP or PLG administered before precipitated abstinence was explained with the prevention of the increase in the BAA.

Intensification of abstinence syndrome Attenuation of abstinence syndrome D-Aspartic acid PLG
Morphine dependence

L-ASPARTIC acid (L-ASP) has been reported to antagonize the effects of morphine (M) such as the inhibition by acute intravenous (IV) M injection and M containing pellets implantation of the brain L-asparaginase activity (BAA) (9,11), the development of physical dependence and the manifestation of abstinence syndrome signs (8, 10, 12). On the basis of these results it has been hypothesized that the development of physical dependence on and the abstinence syndrome upon withdrawal from opiates might be related to a disequilibrium between L-asparaginase and asparagine synthetase due to the inhibitory effect of opiates on L-asparaginase (2,15). The similarity between the effects of M and another L-asparaginase inhibitor (14), D-aspartic acid (D-ASP) (3, 6, 7, 13), and the successful use of L-ASP in the treatment of opiate-addicted persons (2,15), were considered as supporting evidence for the hypothesis.

On the other hand, PLG (prolyl-leucyl-glycinamide, MIF) has been shown to facilitate the development of physical dependence on M (16) and to decrease feeding and drinking as M and D-ASP (6). Additionally, PLG has been found to inhibit the BAA (5) and to provide the release of vasopressin in homozygote diabetes insipidus Brattleboro rats which have an impaired development of tolerance to M analgesia (1). As a consequence, the BAA found higher than normal in homozygote diabetes insipidus Brattleboro rats has been claimed to be the reason of hereditary diabetes insipidus and the impaired development of the tolerance to M analgesia (4).

In the light of the experimental results mentioned above, it was thought it would be of interest to inhibit the BAA by the inhibitors D-ASP or PLG at the beginning of the M physical development or just before the precipitated abstinence syndrome in order to see the probable intensifying or suppressing effect of the BAA inhibition. If the inhibition of the BAA at the beginning of physical dependence development, or before the precipitated abstinence, can respectively increase or decrease the precipitated abstinence syndrome intensity, the hypothesis regarding the relationship between the development of opiate physical dependence and the inhibition of the BAA will experimentally be supported once again.

METHOD

BALB/c mice and inbred *Rattus norvegicus albus* were used for the determination of the precipitated abstinence syndrome intensity and the BAA, respectively. D-ASP and PLG were purchased from Sigma (St. Louis). Naloxone (NL) was a gift from Endo Laboratories (New York).

All the mice and rats were kept in a room at 22–23°C on 12-hour light/dark cycle and fed with a standard regimen ad lib. All the animals had free access to drinking water throughout the experiments.

The BAA was estimated according to the method described in Worthington Enzyme Manual (18). Two g % of D-ASP solution was prepared in bidistilled water. Its pH and osmolality were adjusted to 7.4 and 290 mOsm/kg with 1 N·NaOH and NaCl, respectively. The 2.5 g % PLG solutions were freshly prepared in physiological saline.

One hundred and ten BALB/c mice weighing 25–30 g were divided into six groups, 20 mice in the first four groups and 15 mice in the last two groups. With the exception of the mice belonging to the first group (Control) all the mice of the remaining groups were subcutaneously (SC) implanted with one pellet containing 75 mg "morphine base" (17) on the back of the animals under light ether anesthesia. The mice belonging to Control group were implanted with one pellet prepared without M. The mice of two groups consisted of 20 animals were injected with either 200 mg/kg D-ASP or 25 mg/kg PLG intraperitoneally (IP) just after recovery from anesthesia. The others were IP administered the same volume of physiological saline instead. These treatments were repeated twice, six and twelve hours following the first ones. The groups which had been given D-ASP or PLG were called D-ASP+M or PLG+M groups.

Seventy-two hours after the pellet implantation, two groups of mice, which had had neither D-ASP nor PLG before, were given IP 200 mg/kg D-ASP or 25 mg/kg PLG. The other groups received IP the same volume of physiological saline. The groups which had been administered D-ASP or PLG on the third day were called M+D-ASP (Morphine+D-ASP) and M+PLG (Morphine+PLG) groups, respectively. Fifteen min after the administration of either D-ASP or PLG or saline all mice were injected IP with 1 mg/kg NL and they were immediately placed in a beaker (height 20 cm). During the observation period of 15 min the number of some countable precipitated abstinence syndrome signs such as flying, jumping, wet dog shake and writhing were counted (17).

One hundred fifty rats were divided into six groups, 25 rats in each. The first group was implanted SC with 3 pellets without M, whereas the others were implanted SC with 3 pellets containing 75 mg (in toto 225 mg) "morphine base" (17) on the back of the animals under light ether anesthesia. After recovery from anesthesia two groups of rats were administered IP either 200 mg/kg D-ASP or 25 mg/kg PLG and the administration of D-ASP or PLG were repeated twice six and twelve hours following the first ones. The remaining other three groups were IP given the same volume of physiological saline. On the third day, 72 hours after pellet implantation, two groups of rats which had been implanted with M-containing pellets and then given physiological saline were injected IP with either 200 mg/kg D-ASP or 25 mg/kg PLG, respectively. The others were administered IP saline. Fifteen min following the injections half of the rats from each group taken at random received IP 10 mg/kg NL, whereas the remaining halves were given IP the same volume of saline. Five min following the administrations the rats were decapitated after cervical dislocation. After the brains had immediately been removed and cleaned of extraneous tissues in the containers chilled on ice they were weighed. For the determination of the BAA the same procedures and methods used elsewhere (3) were carried out and the activity of L-asparaginase was expressed as IU/g wet weight of the brain (one international unit is the activity which catalyzes the release of one micromole of ammonia from asparagine per minute at 37°C and pH 8.6).

The rats implanted with pellets not containing M and injected with either physiological saline or NL on the third day consisted of Control and Naloxone (N) group, respectively. The remaining groups which had had different treatments after three morphine-containing pellets were called as follows:

On the 1st Day	On the 3rd Day	Symbols
Saline	+ Saline + Saline	M
Saline	+ Saline + NL	M+N
D-ASP	+ Saline + Saline	D-ASP+M
D-ASP	+ Saline + NL	D-ASP+M+N
PLG	+ Saline + Saline	PLG+M
PLG	+ Saline + NL	PLG+M+N
Saline	+ D-ASP + Saline	M+D-ASP
Saline	+ D-ASP + NL	M+D-ASP+N
Saline	+ PLG + Saline	M+PLG
Saline	+ PLG + NL	M+PLG+N

All the results of flying, jumping, wet dog shake, writhing and BAA were separately analyzed by analysis of variance and subsequently the Dunnett's (I) test was used for statistical evaluation.

RESULTS

The effects of D-ASP or PLG administration just after the M-containing pellet implantation and 15 min before the IP NL injection on the observed precipitated abstinence syndrome signs are shown in Table 1. D-ASP or PLG given at the beginning of the development of physical dependence significantly increased the number of flying, jumping, wet dog shake and writhing in the animals received D-ASP or PLG just after the implantation of M pellet. Control group did not show any of the observed abstinence syndrome signs. On the other hand, when D-ASP or PLG were injected to the mice implanted with M pellet 15 min before the onset of precipitated abstinence syndrome, they brought about a significant decrease in the observed abstinence syndrome signs, namely in flying, jumping, wet dog shake and writhing (Table 1).

The BAA of the rats implanted with either M-containing or not-M-containing pellets and treated with D-ASP or PLG or NL in variable combinations, and their statistical evaluation can be seen in Table 2. The administration of NL showed no significant effect on the BAA, whereas M decreased the BAA significantly. The increase by NL in the BAA of the M-containing pellet-implanted rats exceeded the control value significantly (Table 2).

The BAA of the rats received D-ASP or PLG just after pellet implantation was found significantly lower than control (D-ASP+M and PLG+M groups). The administration of NL to the rats treated in the same manner resulted in a significant increase more than control (D-ASP+M+N and PLG+M+N groups). When the rats implanted with M-containing pellets were injected with D-ASP or PLG on the third day of the implantation (M+D-ASP and M+PLG groups) the BAA appeared significantly lower than control and it did not show any significant change compared to that of the rats implanted with M-containing pellets (M group). But the injection of NL, which brought the BAA from the significantly lower levels than control to the significantly higher levels in D-ASP+M+N and PLG+M+N groups, could only normalize in M+D-ASP+N and M+PLG+N groups (Table 2). The statistically significant differences between M and M+N, D-ASP+M and D-ASP+M+N, PLG+M and PLG+M+N, M+D-ASP and M+D-ASP+N, M+PLG and M+PLG+N, M+N and

TABLE 1

THE MEAN VALUES OF THE NUMBERS OF FLYING, JUMPING, WET DOG SHAKE AND WRITHING AS NALOXONE-PRECIPITATED ABSTINENCE SYNDROME SIGNS AND THEIR STANDARD DEVIATIONS IN THE MICE AFTER THE IMPLANTATION OF EITHER MORPHINE-CONTAINING OR NONMORPHINE-CONTAINING PELLET AND THE DIFFERENT TREATMENTS WITH D-ASP OR PLG

Groups	Flying	Jumping	Wet Dog Shake	Writhing
Control (17)	0	0	0	0
M (16)	18.81 ±5.83	34.50 ±6.05	11.87 ±4.27	4.31 ±1.88
D-ASP+M (13)	32.07* ±7.33	66.07* ±15.11	23.30* ±5.89	9.61* ±3.04
PLG+M (11)	36.54* ±6.26	76.09 ±15.57	26.09* ±4.45	11.27* ±2.61
M+D-ASP (11)	7.81* ±3.42	17.54* ±3.04	3.45* ±2.06	1.36* ±1.28
M+PLG (12)	6.76* ±2.35	14.41* ±3.08	3.23* ±1.87	1.46* ±1.26
F-value	2.79	3.04	2.84	2.63

Control—Vehicle-containing pellet-implanted group.
M—Morphine-containing pellet-implanted group.
D-ASP+M—Just after pellet implantation D-aspartic acid administered group.
PLG+M—Just after pellet implantation PLG administered group.
M+D-ASP—Fifteen min before naloxone injection D-aspartic acid given group.
M+PLG—Fifteen min before naloxone injection PLG given group.
*→$p<0.001$ statistical significance referring to the values of the M group.
The figures in parentheses indicate the number of the animals in the group.

M+D-ASP+N, and finally M+N and M+PLG+N can be useful in showing the significant increase by NL in the BAA of the abstinent animals.

DISCUSSION

The countable precipitated abstinence syndrome signs such as flying, jumping, wet dog shake and writhing have almost unanimously been considered as the signs most reliable and informative especially in comparative studies due to their objectivity.

The animals which had received D-ASP or PLG just after M-containing pellet implantation showed significantly more flying, jumping, wet dog shake and writhing as precipitated abstinence syndrome signs than the animals which had not been given D-ASP or PLG (Table 1). These results can naturally be considered as supporting evidence for the fact that PLG facilitates M tolerance and dependence (16) and that the inhibition of the BAA can be one of the main reasons of the M physical dependence development (2,15). As both D-ASP and PLG inhibit L-asparaginase (5,14) as does M, and they have similar effects to those of M (3–7, 13), the significant increase in the number of the abstinence syndrome signs in the animals administered D-ASP or PLG at the beginning of the physical dependence development can clearly be attributed to the effects of D-ASP or PLG. On the other hand, the BAA of the rats implanted with M pellets was found significantly lower than control and the injection of NL to these rats caused an increase reaching significantly over the control value. This can naturally bring about many changes in the levels of neurotransmitters and hormones which play a role in the manifestation of abstinence syndrome.

The BAA of the animals implanted with M-containing pellets and then immediately administered D-ASP or PLG does not seem to have a decrease deeper than that of the animals which did not have D-ASP or PLG. Additionally, the BAA of the animals which were implanted with pellets and given D-ASP or PLG just after the pellet implantation is not significantly higher than the BAA of the animals which did not receive D-ASP or PLG following M pellets

TABLE 2

THE MEAN VALUES OF THE WHOLE BRAIN L-ASPARAGINASE ACTIVITY (IU/g WET WEIGHT) AND THEIR STANDARD DEVIATIONS IN THE RATS AFTER THE IMPLANTATION OF EITHER MORPHINE-CONTAINING OR NONMORPHINE-CONTAINING PELLETS AND THE DIFFERENT TREATMENTS WITH D-ASP OR PLG OR NALOXONE

Control (11)	N (11)	M (12)	M+N (11)	D-ASP+M (11)	D-ASP+M +N (10)	PLG+M (11)	PLG+M +N (11)	M+D− ASP (10)	M+D− ASP+N (11)	M+PLG (11)	M+PLG +N (12)	F-Value
0.139 ±0.012	0.145 ±0.012	0.120† ±0.008	0.162† ±0.009	0.117† ±0.014	0.161† ±0.017	0.115† ±0.007	0.159† ±0.008	0.123* ±0.012	0.141 ±0.017	0.125† ±0.005	0.146 ±0.010	2.91

Control—Vehicle-containing pellet implanted group.
N—Naloxone-injected "Control" group.
M—Morphine-containing pellet-implanted group.
M+N—Naloxone-injected "M" group.
D-ASP+M—Just after pellet implantation D-aspartic acid administered group.
D-ASP+M+N—Naloxone-injected "D-ASP+M" group.
PLG+M—Just after pellet implantation PLG administered group.
PLG+M+N—Naloxone-injected "PLG+M" group.
M+D-ASP—Fifteen min before naloxone injection D-aspartic acid given group.
M+D-ASP+N—Naloxone-injected "M+D-ASP" group.
M+PLG—Fifteen min before naloxone injection PLG given group.
M+PLG+N—Naloxone-injected "M+PLG" group.
*→$0.01<p<0.001$ referring to control; †→$p<0.001$ referring to control.
The statistical significance between: M and MN; D-ASP+M and D-ASP+M+N; M+D-ASP and M+D-ASP+N; M+PLG and M+PLG+N; M+N and M+D-ASP+N; M+N and M+PLG+N is $p<0.001$.
The figures in parentheses indicate the number of rats in the group.

implantation (Table 2). Since the physical dependence of the animals belonging to D-ASP+M+N and PLG+M+N groups showed significantly more intense abstinence syndrome signs than M+N group, one would expect that the inhibition of the BAA might have been stronger in D-ASP+M+N or PLG+M+N groups than in M+N group if the hypothesis works. But D-ASP or PLG were administered on the first day of the physical dependence development, instead the determination of the BAA was carried out on the third day. When D-ASP or PLG were given at the beginning of the opiate physical dependence development, BAA was already inhibited before the BAA inhibiting effect of M started. As M found BAA already inhibited by D-ASP or PLG, the inhibiting effect of M and the course of the inhibition strengthened and extended. These naturally intensified physical dependence and abstinence syndrome. In the end, the difference between the severities of abstinence syndrome in M+N, and D-ASP+M+N and PLG+M+N groups was found (Table 1).

The administration of D-ASP or PLG just before challenging the M-dependent animals with NL caused a significant decrease in all the observed countable abstinence syndrome signs (Table 1), and the BAA which had been found inhibited in the animals of M group was more or less at the same level in the brain of the animals received D-ASP or PLG before NL administration. But NL could not bring this inhibited BAA significantly over the control value (Table 2); it only caused the normalization of the inhibited activity of the enzyme under the inhibitory effect of D-ASP or PLG on L-asparaginase (Table 2). As a result, the manifestation of the precipitated abstinence syndrome in M+D-ASP+N and M+PLG+N groups was not as strong as in M+N group since the increases in the activity of L-asparaginase in M+D-ASP+N and M+PLG+N groups were not as great as in M+N group.

Even though the inhibition and disinhibition of the BAA can, to a greater extent, explain the intensifying and attenuating effect of D-ASP or PLG given at the beginning of the physical dependence development and 15 min before precipitated abstinence syndrome, respectively, at least two points remain to be elucidated: The determination of the BAA after D-ASP or PLG administrations on the first day in order to find supporting evidence for the fact that the administration of D-ASP or PLG + the implantation of M pellets could inhibit the enzyme more than the M pellets implantation alone, and the investigation of the ineffectiveness of D-ASP or PLG administration for the additional inhibition of the enzyme in M+D-ASP and M+PLG groups before NL injection.

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