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Vasopressin Release by D-Aspartic Acid, Morphine and Prolyl-leucyl-glycinamide (PLG) in DI Brattleboro Rats

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KOYUNCUOĞLU, H., K. BERKMAN, I. HATIPOĞLU AND H. SABUNCU. *Vasopressin release by D-aspartic acid, morphine and prolyl-leucyl-glycinamide (PLG) in DI Brattleboro rats.* PHARMACOL BIOCHEM BEHAV **20**(4) 519–525, 1984.—The L-asparaginase activities of the brains of the Wistar, heterozygous and homozygous Brattleboro rats divided into three parts namely the anterior, middle and posterior which respectively contained cerebral cortex, hippocampus + midbrain + thalamus + hypothalamus and cerebellum + pons + medulla oblongata were estimated. The L-asparaginase activities of all the three parts in the homozygous Brattleboro rats were significantly higher than in the Wistar rats as well as in the heterozygous Brattleboro rats. Twenty min following the injections of 200 mg/kg D-aspartic acid, 20 mg/kg morphine, 200 mg/kg D-aspartic acid + 20 mg/kg morphine, 6 mg/kg prolyl-leucyl-glycinamide (PLG) and 6 mg/kg PLG + 20 mg/kg morphine the L-asparaginase activities of all three parts of the homozygous Brattleboro rat brains were found to be significantly inhibited. After having seen the suppressive effect of the drugs and their combinations used before the homozygous Brattleboro rats were given D-aspartic acid, morphine, D-aspartic acid + morphine, PLG and PLG + morphine for seven days. Then their plasma vasopressin levels were determined by RIA. The treatments applied to the homozygous Brattleboro rats caused the appearance of a significant amount vasopressin in the plasma. The results were interpreted as evidence for the fact that the inhibition of the brain L-asparaginase provides and/or accelerates the biosynthesis and/or release of vasopressin. As morphine has a vasopressin releasing and a brain L-asparaginase inhibiting effect the antidiuretic action of morphine was considered to be linked to its inhibitory effect on the brain L-asparaginase.

Vasopressin release DI Brattleboro rats D-Aspartic acid Morphine PLG L-Asparaginase

IN our previous studies we found that the levels of free amino acids in the brains of the rats rendered physically dependent on morphine were significantly higher than normal [24,26] and that a further increase occurred after abrupt withdrawal from morphine [24,28] as well as after the administration of a morphine antagonist [28]. The results strongly suggested that free amino acids in the CNS played an important role in the mechanism of action of morphine. On these grounds we tried to antagonize the effects of morphine by using L-aspartic acid. We showed that L-aspartic acid antagonized the effects of acute and chronic administration of morphine in rats [23, 25–30]. Encouraged by the results obtained from the experiments performed on animals L-aspartic acid was successfully used for the treatment of persons addicted to opiates without hospitalizing and withdrawing them from drugs. They gradually attenuated the compulsory drug intake and at the end of the treatment they did not show any need to take any kind of narcotics [22]. As morphine has an inhibitory effect on the brain L-asparaginase activity which is also antagonized by L-aspartic acid [30] it has been hypothesized that the development of physical dependence on, and the abstinence syndrome upon withdrawal from opiates, may be related to a disequilibrium between L-asparaginase and asparagine synthetase, caused by the inhibitory effect of opiates on the activity of L-asparaginase. These two enzymes regulate the biosynthesis of peptides and proteins containing aspartic acid and asparagine. These processes are extremely important for the formation of glycopeptides and glycoproteins, and also for the adaptation of the organism to the consequent biochemical and behavioural alterations resulting from the disequilibrium. The therapeutic action of L-aspartic acid has been attributed to its direct and indirect effects, first on L-asparaginase and then on asparagine synthetase. L-aspartic acid, being an end product of the L-asparaginase activity shows a short-lasting inhibitory effect on the activity of the enzyme (this inhibitory effect was shown in vitro, our unpublished observation) which may, to some extent, result in the attenuation of narcotic intake since a sufficient inhibition of L-asparaginase, to which the organism has been adapted, is necessary. Following this inhibition it is assumed that L-aspartic acid is converted into asparagine via the activity of asparagine synthetase which seems to be found in relatively higher quantities in opiate-dependent subjects due to the inhibition of L-asparaginase. Once L-aspartic acid is converted into asparagine, asparagine as an end product of

asparagine synthetase begins to inhibit the enzyme. In the end the equilibrium altered by the chronic intake of the opiate between L-asparaginase and asparagine synthetase and the suppressed activity of L-asparaginase are supposed to be restored and normalized. Such restoration and normalization of the functions of these two enzymes abolish the need to take the drug [22].

In order to provide some more experimental findings in favour of the hypothesis mentioned above we used D-aspartic acid which had been reported to be an inhibitor of L-asparaginase [35] as morphine [30]. The oral administration of D-aspartic acid caused a decrease in food and fluid intakes, urine outflow and naloxone reversible decrease in rectal temperature, and an increase in the osmolality of urine, the brain levels of free amino acids in normal rats. L-aspartic acid antagonized all these effects of D-aspartic acid as it did antagonize those of morphine ([31, 32, 33] Koyuncuoğlu and Berkman, *Ist.Tip Fak.Med.* in press). When the effects of the parenteral administration of D- and L-aspartic acids, morphine, naloxone, D-phenylalanine (an inhibitor of carboxypeptidase A) and D-leucine (an inhibitor of leucine aminopeptidase) which produce opioid-like analgesia through endorphinergic system [7, 15, 16], and PLG (the C-terminal tripeptide of oxytocin, prolyl-leucyl-glycinamide, MIF) which facilitates morphine dependence [40] on food and fluid intakes, urine outflow, urine osmolality and rectal temperature were investigated in homozygous Brattleboro rats sufferring from severe diabetes insipidus, D-aspartic acid, morphine and PLG exerted similar effects on homozygous Brattleboro rats to those obtained with D-aspartic acid and morphine in normal rats. The effects of D-phenylalanine and D-leucine were completely different from those of D-aspartic acid, morphine and PLG the majority of which were antagonized by L-aspartic acid. Since the increase by D-aspartic acid, morphine and PLG in urine osmolality, reaching up to 343,340 and 316 mOsm/kg respectively were over the osmolality of normal plasma (280 mOsm/kg) the probability of the vasopressin release was thought: because the urine osmolality of the subjects with idiopathic diabetes insipidus is about 100 to 200 mOsm/kg unless a severe water lack which may increase the osmolality only up to 300 mOsm/kg exists [10]. As only D-aspartic acid, morphine and PLG among the drugs used in the experiments (Koyuncuoğlu *et al. Pharmacol Biochem Behav* in press) inhibit the activity of the brain L-asparaginase (Koyuncuoğlu and Berkman, Med Bull Ist Med Fac, in press) the mechanism underlying the probable vasopressin release in the Brattleboro rats sufferring from diabetes insipidus was assumed to be the inhibition of the brain L-asparaginase activity (Koyuncuoğlu *et al. Pharmacol Biochem Behav,* in press).

As known homozygous Brattleboro rats which lack the ability to synthetize and/or release vasopressin have difficulty in developing tolerance to and dependence on narcotics. This process has been attributed to the important role of vasopressin in learning and memory consolidation which are extremely related to the protein biosynthesis [1, 12, 34]. On the other hand it has been reported that Brattleboro homozygous diabetes insipidus rats have an impaired body and brain growth, and the protein content of their brains is lower than those of the different controls [3]. All these experimental findings suggest the existence of an altered protein and peptide biosynthesis in the brain as well as in the whole body of the homozygous Brattleboro rats for which the hyperactivity of L-asparaginase seems to be the most probable candidate as L-asparaginase has long been used for the treatment of acute leukemias and lymphomas because of its asparagine depleting effect by deamidation of asparagine [4, 5, 11, 14].

Taken together all the information given above we thought it would be of interest to determine the brain L-asparaginase activity of homozygous Brattleboro rats in comparison with those of normal and heterozygous Brattleboro rats, to further show the inhibitory effect of D-aspartic acid, morphine and PLG also on the brain L-asparaginase activity of homozygous Brattleboro rats, and to finally investigate the effects of long-term administration of D-aspartic acid, morphine and PLG on homozygous Brattleboro rats in order to see whether they would be able to create a suitable condition for the biosynthesis and/or release of vasopressin. If D-aspartic acid, morphine and PLG inhibited also the brain L-asparaginase activity in homozygous Brattleboro rats which had been supposed to be found higher than in normal rats as well as in heterozygous Brattleboro ones, and a detectable amount of vasopressin were found after the inhibition our hypothesis concerning the mechanism of the development of physical dependence on narcotics would be supported by some more experimental findings and the following points remained obscure so far would, to a great extent, be clarified.

Can the mechanism underlying the idiopathic diabetes insipidus simply be the hyperactivity of the brain L-asparaginase?

May the hyperactivity of the brain L-asparaginase be the main reason of the impaired development of physical dependence on and tolerance to opiates in homozygous Brattleboro rats?

Does morphine cause the release of vasopressin by inhibiting the brain L-asparaginase?

Why and how does the single dose of PLG facilitate the development of physical dependence on narcotics?

METHOD

To see whether the brain L-asparaginase activity of homozygous Brattleboro rats is higher than those of Wistar and heterozygous Brattleboro rats and to show D-aspartic acid, morphine and PLG are capable to inhibit the brain L-asparaginase activity also in homozygous Brattleboro rats 20 male Rattus norvegicus var. albinos Wistar (The Experimental Research Institute, Istanbul Med Fac/Istanbul), 20 male heterozygous and 120 homozygous Brattleboro rats (inbred in our laboratories from the heterozygous and homozygous Brattleboro rats originally provided by Centraal Proefdierenbedrijf, Zeist/Holland) weighing 200–220 g were used. The 20 Wistar, 20 heterozygous and 20 homozygous Brattleboro rats were injected with 0.5 ml/kg of physiological saline subcutaneously (SC) and 10 ml/kg of 5 g % glucose solution intraperitoneally (IP). They were called the Wistar rats, heterozygous Brattleboro rats and homozygous Brattleboro rats groups, respectively. The remaining 100 homozygous Brattleboro rats were divided into five groups, 20 rats in each. The first (the Homozygous Brattleboro rats-D-aspartic acid), the second (the Homozygous Brattleboro rats-morphine), the third (the Homozygous Brattleboro rats-D-aspartic acid + morphine), the fourth (the Homozygous Brattleboro rats-PLG) and the fifth (the Homozygous Brattleboro rats-PLG + morphine) groups were received 0.5 ml/kg physiological saline SC + 10 ml/kg of 2 g % D-aspartic acid solution (200 mg/kg), 0.5 ml/kg of 4 g % morphine solu-

tion SC (20 mg/kg) + 10 ml/kg of 5 g % glucose solution IP, 0.5 ml/kg of 4 g % morphine solution SC + 10 ml/kg of 2 g % D-aspartic acid solution IP, 0.5 ml of physiological saline SC + 6 ml/kg of 0.1 g % PLG in 5% glucose solution IP (6 mg/kg) and 0.5 ml/kg of 4 g % morphine solution SC + 6 ml/kg of 0.1 g % PLG solution IP, respectively. Twenty min following the injections the rats were decapitated after cervical dislocation. Their brains were immediately removed and collected in containers chilled on ice. After they had been cleaned of extraneous tissues they were cut into three parts namely the anterior, middle and posterior parts provided they contained cerebral cortex, hippocampus + midbrain + thalamus + hypothalamus and cerebellum + pons + medulla oblongata, respectively [17]. Because of the insufficiency of every single brain part to yield the necessary amount of homogenate two same parts of the different two brains were considered as one sample reducing their numbers from 20 to 10. After weighing the samples they were homogenized by means of a glass Potter-Elwehjem homogenizer with teflon pestle in 4 times the volume of the sample weight in 0.05 M Tris-HCl pH 8.6 chilled on ice. L-Asparaginase activity was determined by measuring ammonia liberated from asparagine [2,37]. A "test" tube containing 0.2 ml of 0.05 M Tris-HCl pH 8.6 and 1.8 ml of 0.10 M L-asparagine and a "blank" tube containing 0.5 ml of 3 M trichloroacetic acid (TCA) in addition to the content of the "test" tube were prepared and incubated at 37° for 5–6 min to achieve temperature equilibration. At zero time and at timed intervals 1 ml of homogenate was added into the "test" and "blank" tubes. They were incubated at 37°C for exactly 30 min and the reaction was stopped by adding 0.5 ml of 3 M TCA to the "test" tube only. After centrifugation of the tubes 1 ml of the clear supernatants was added into 5 ml glass distilled water. After adding 1 ml of Nessler's reagent and incubating at room temperature for 10 min the "test" tube's was read (A425) versus the respective blank. The amount of ammonia liberated was determined from an ammonia sulfate standard curve and the activity of L-asparaginase was expressed as IU/g wet weight (One international unit is the activity which catalyzes the release of one micromole of ammonia per minute at 37°C and pH 8.6).

To investigate the probable effect of D-aspartic acid, morphine and/or PLG on the release of vasopressin 60 male homozygous Brattleboro rats (Centraal Proefdierenbedrijf, Zeist/Holland) weighing 250–280 g were divided into six groups, 10 rats in each. Before the administration of the drugs they were given 1 g % sucrose solution instead of drinking water for two days. The first (Control), the third (Morphine), the fifth (PLG) and the sixth (PLG + Morphine) groups went on drinking 1 g % sucrose solution whereas the second (D-Aspartic acid) and the fourth (D-Aspartic acid + Morphine) groups drank 1 g % sucrose solution containing 0.5 g % D-aspartic acid for another seven days. The fluid consumed daily by each rat was measured. The rats were kept in a room at 22–23°C on a 12 hours light/dark cycle and they were fed with a standard regime ad lib throughout.

The rats belonging to the Control and D-Aspartic acid groups received one SC and one IP injection of 0.5 ml physiological saline twice a day (at 9.00 a.m. and 3.00 p.m.). The Morphine and D-Aspartic acid + Morphine groups were injected with 0.5 ml physiological saline IP and 10 mg/kg morphine in 0.5 ml physiological saline SC, twice a day. The PLG group were given 0.5 ml physiological saline SC and 3 mg/kg PLG in 0.5 ml physiological saline IP at 9.00 a.m., and two 0.5 ml physiological saline, one SC the other IP, at 3.00 p.m. Finally, the PLG + Morphine group received one IP injection of PLG and one SC injection of 10 mg/kg morphine, both in 0.5 ml physiological saline, at 9.00 a.m., and one IP injection of 0.5 ml physiological saline and one SC injection of 10 mg/kg morphine at 3.00 p.m. The doses of morphine and PLG were doubled in the last applications at the end of the administration period: The Morphine, D-Aspartic acid + Morphine, PLG and PLG + Morphine groups were injected with 20 mg/kg morphine, 6 mg/kg PLG and 6 mg/kg PLG + 10 mg/kg morphine, respectively. One hour following the last injections the rats were weighed and then decapitated after cervical dislocation. Their bloods were collected in polypropylene test tubes containing EDTA chilled on ice. After centrifugation at 0°C their plasma were removed into polyproplyene test tubes and frozen in liquid nitrogen. They were kept at −70°C until the determinations.

One rat from the Morphine and PLG + Morphine groups, and two rats from the D-Aspartic acid + Morphine and PLG groups died during the administration period.

For the extraction of vasopressin from plasma the procedures used elsewhere [38] were carried out. Briefly, one ml aliquots of plasma were transferred into polypropylene test tubes containing 0.5 ml of 1 N HCl and mixed thoroughly. Three mg of Bentonite in 1 ml deionized water were added to each sample of acidified plasma and mixed for 20 min at room temperature. The mixture was centrifuged at 2000 × g for 30 min and the supernatant removed. One ml of an 80% solution of acetone in 1 N HCl was added onto the pellet which was vigorously agitated for 30 sec to remove the absorbed vasopressin. The Bentonite suspension was again centrifuged at 2000 × g for 10 min and the supernatant acetone was transferred into a polypropylene tube and then 0.2 ml deionized water and 1 ml ether were added. After thorough mixing, the solution was allowed to separate into an upper and lower phase and the upper phase was removed and discarded. The lower aqueous phase was evaporated to dryness by a gentle stream of compressed air and redissolved in 1 ml standard buffer. Two 0.4 ml aliquots equivalent to 0.4 ml plasma were then assayed by following strictly the procedures described in the booklet of the "arginine vasopressin by radioimmunoassay kit (Immuno Nuclear Corporation, Stillwater, MN, Cat. No. 23L1) which is based on the using of double antibody.

The doses of morphine and PLG administered to the rats were kept constant throughout the experiments in spite of the changes in body weight.

D-Aspartic acid and PLG were purchased from Sigma (St. Louis/USA), morphine from Sandoz (Basle/Switzerland).

All the results were analyzed by analysis of variance and subsequently Bonferroni t statistics were calculated for each comparison. For finding the statistical significance Bonferroni/Dunn tables were used.

RESULTS

The mean values of the L-asparaginase activities (expressed as IU/g wet weight) of the brain anterior, middle and posterior parts, and their statistical evaluation can be seen in Table 1. The L-asparaginase activities of the brain anterior, middle and posterior parts in the heterozygous and homozygous Brattleboro rats were significantly higher than those in the Wistar rats. When the L-asparaginase activities of these three parts of the homozygous Brattleboro rats were compared with those of the heterozygous Brattleboro rats the L-asparaginase activities of the heterozygous ones were found significantly lower (Table 1). The L-asparaginase ac-

TABLE 1

Groups	Anterior part	Middle part	Posterior part
Wistar rats (10)	0.065 ± 0.004	0.056 ± 0.004	0.045 ± 0.003
Heterozygous Brattleboro rats (10)	0.096 ± 0.006*	0.098 ± 0.006*	0.067 ± 0.005*
Homozygous Brattleboro rats (10)	0.150 ± 0.005*†	0.118 ± 0.005*†	0.105 ± 0.005*†
Homo. Brattleboro rats D-aspartic acid (10)	0.101 ± 0.004‡	0.083 ± 0.004‡	0.068 ± 0.003‡
Homo. Brattleboro rats morphine (10)	0.103 ± 0.004‡	0.086 ± 0.005‡	0.066 ± 0.003‡
Homo. Brattleboro rats D-aspartic acid + morphine (10)	0.084 ± 0.003‡§	0.073 ± 0.003‡§	0.059 ± 0.003‡§
Homo. Brattleboro rats PLG (10)	0.064 ± 0.003‡	0.057 ± 0.03‡	0.053 ± 0.003‡
Homo. Brattleboro rats PLG + morphine (10)	0.064 ± 0.003‡	0.056 ± 0.003‡	0.054 ± 0.003‡
ANOVA testing	$p<0.001$	$p<0.01$	$p<0.01$

The mean values (±SE) of the L-asparaginase activity (expressed as IU/g wet weight) of the brain anterior, middle and posterior parts.

The doses of D-aspartic acid, morphine and PLG were 200 mg/kg, 10 mg/kg and 6 mg/kg, respectively.

The figures in parenthesis indicate the number of the determinations.

*→$p<0.01$ Between the values of the Wistar rats, and those of the heterozygous and homozygous Brattleboro rats.

†→$p<0.01$ Between the values of the heterozygous and homozygous Brattleboro rats.

‡→$p<0.01$ Between the values of the homozygous Brattleboro rats not received any drugs and those of the homozygous Brattleboro rats given D-aspartic acid, morphine, D-aspartic acid + morphine, PLG and PLG + morphine.

§→$p<0.01$ Between the values of the homozygous Brattleboro rats administered D-aspartic acid and D-aspartic acid + morphine. The statistical evaluation between the PLG and the PLG + morphine administered groups showed no significance.

tivities of the three brain parts taken from the homozygous Brattleboro rats treated with D-aspartic acid, morphine, D-aspartic acid + morphine, PLG and PLG + morphine showed a statistically significant decrease when compared to those of the untreated homozygous Brattleboro rats (Table 1). The administration of D-aspartic acid together with morphine appeared to be more effective in decreasing the activity of L-asparaginase than the administration of D-aspartic acid alone. The statistical evaluation between the PLG and the PLG + Morphine groups showed no statistical significance (Table 1).

The mean values of the daily fluid intake per 100 g body weight, the initial and final body weights, and their statistical analyses are shown in Table 2. The fluid intakes of the D-aspartic acid, Morphine, D-Aspartic acid + Morphine and PLG + Morphine groups were statistically lower than that of the control group. The body weights of the D-Aspartic acid, Morphine, D-aspartic acid + Morphine and PLG + Morphine groups estimated before killing the rats showed a statistically significant decrease when compared with their body weights at the beginning of the experiments (Table 2).

The plasma vasopressin levels (expressed as pg/ml) of all the groups were statistically higher that of the control group which was absolutely null (Table 3). When the plasma vasopressin level of the D-Aspartic acid + Morphine group was compared with that of the D-Aspartic acid group the former appeared to be statistically higher. The statistical analysis between the PLG and the PLG + Morphine groups did not show any statistical significance (Table 3).

DISCUSSION

The plasma vasopressin level of the control group was found to be undetectable as expected. All the treatments applied to the homozygous Brattleboro rats made a remarkable amount of vasopressin appear in the plasma (Table 3).

TABLE 2

Groups	Fluid Intake (ml/day/100 g BW)	Initial Body Weight (g)	Final Body Weight (g)
Control (10)	51.16 ± 0.92	272.17 ± 1.36	276.44 ± 1.79
D-Aspartic acid (10)	33.26 ± 0.79*	277.00 ± 0.44	248.21 ± 1.65§
Morphine (9)	43.65 ± 1.09*	255.70 ± 2.23	235.69 ± 2.28‡
D-Aspartic acid + Morphine (8)	45.31 ± 0.89*†	258.10 ± 0.74	234.38 ± 2.16§
PLG (8)	51.21 ± 0.97	284.00 ± 2.34	276.25 ± 1.99
PLG + Morphine (9)	39.61 ± 0.70*†	284.20 ± 1.03	259.00 ± 2.09§
ANOVA testing	$p<0.01$		

The mean values (±SE) of the daily fluid intake per 100 g body weight, initial and final body weights of the homozygous Brattleboro rats and their statistical evaluations.

The figures in parenthesis indicate the number of the rats.

*→$p<0.01$ Between the fluid intakes of the Control and the other groups.

†→$p<0.01$ Between the fluid intakes of the D-Aspartic acid and the D-Aspartic acid + Morphine groups, and of the PLG and the PLG + Morphine groups.

‡→$p<0.05$, §→$p<0.01$ Between the initial and final body weights in each group.

Even though the most unfavourable situations are assumed with respect to the missing values of the rats which died during the administration period the plasma vasopressin levels of the Morphine, D-Aspartic acid + Morphine, PLG and PLG + Morphine groups remain statistically significant in comparison with the control plasma vasopressin level. Since the antibody to vasopressin used in radioimmunoassays has very low cross reactivity with oxytocin and vasotocin (<0.01% and 0.14%, respectively) there should not be any doubt about the appearance of vasopressin in the plasma of the hymozygous Brattleboro rats treated with D-aspartic acid, morphine and/or PLG. The results concerning the determinations of the brain L-asparaginase activities in the Wistar, heterozygous and homozygous Brattleboro rats show that the L-asparaginase activities of the three different brain parts in the homozygous Brattleboro rats are considerably higher than those in the Wistar rats as well as those in the heterozygous Brattleboro rats (Table 1). Additionally D-aspartic acid, morphine, D-aspartic acid + morphine, PLG and PLG + morphine which have been shown to have a suppressing effect on the brain L-asparaginase activity in Wistar rats (Koyuncuoğlu and Berkman, *Med Bull Ist Med Fac*, in press) inhibit the activity of L-asparaginase in all the three parts of the homozygous Brattleboro rat brains (Table 1) in a manner indicating that the level of plasma vasopressin is related to the extent of the inhibition of the brain L-asparaginase activity (Table 1 and 3).

Before discussing the results related to fluid intake it would be noted that the differences between the groups with respect to the initial body weights cannot considerably influence the fluid intake/100 g body weight especially in adult homozygous Brattleboro rats suffering from severe diabetes insipidus since fluid intake occurs in response to homeostatic demands implying whole body. Vasopressin, as well estab-

TABLE 3

Groups	The Plasma AVP Levels (pg/ml)
Control (10)	0
D-Aspartic acid (10)	0.928 ± 0.064*
Morphine (9)	0.980 ± 0.071*
D-Aspartic acid + Morphine (8)	1.568 ± 0.164*†
PLG (8)	3.282 ± 0.377*
PLG + Morphine (9)	3.393 ± 0.409*
ANOVA testing	$p<0.001$

The plasma AVP levels (pg/ml) (±SE) of the homozygous Brattleboro rats chronically administered D-Asp and/or morphine, PLG and/or morphine.

The figure in parentheses show the number of the rats.

*→$p<0.01$ Between the Control and the other groups.

†→$p<0.01$ Between the D-Aspartic acid and the D-Aspartic acid + Morphine groups. The statistical evaluation between the PLG and the PLG + Morphine groups was found insignificant.

lished, effects drinking behaviour mainly be reducing urine outflow via the increase in renal tubular water reabsorption. But this is not certainly the unique regulating factor of thirst particularly in homozygous Brattleboro rats which have been reported to have a high angiotensin converting enzyme activity in neurohypophysis [8]. This high activity of angiotensin converting enzyme in homozygous Brattleboro rats may play an important role in polydipsia since captopril, an inhibitor of angiotensin converting enzyme, which prevents the conversion of angiotensin I to a very potent dipsogenic angiotensin II, reduces the polydipsia of homozygous Brattleboro rats by 18% with no change in plasma osmolality [39]. In addition to these some more factors such as neurochemical transmitters and hormones influencing fluid intake may take part in drinking behaviour. Therefore an absolute parallelism between the plasma level of vasopressin and daily fluid intake should not be expected particularly in this rat strain under the conditions of the experiments. However, the failure of PLG in inhibiting the fluid intake and in decreasing body weight can be explained by the fact that the degradation of the peptide is rather rapid [13]. As the rats were given PLG once a day on purpose in the present study the rats could have had a period of time long enough to compensate for the suppressed drinking and eating. In fact when PLG was administered three times in a seven hours period of time it significantly decreased food and fluid intake, urine volume and increased urine osmolality (Koyuncuoğlu *et al. Pharmacol Biochem Behav,* in press). As known "pro-opiomelanocortin" was clearly identified as a multipotent precursor in the rat, producing beta-MSH, adrenocorticotropin (ACTH), beta-lipotropin, alfa-MSH and beta-endorphin [9]. Furthermore a common precursor of adrenocorticotropin and beta-endorphin was found in the hypothalamo-neurohypophyseal tract and was called "coenophorin;" it was a high molecular weight form and contained both neurophysin and vasopressin [41]. In favour of the concomitant secretion of adrenocorticotropin and beta-endorphin it has recently been reported that the N-terminal like immunoreactive material of pro-opiomelanocortin is also changing in the circulation with adrenocorticotropin and beta-endorphin [36]. Additionally it has been shown that vasopressin causes a hyperglycemia [6, 20, 21] probably due to the degradation of liver glycogen as vasopressin activates glycogen phosphorylase and inhibits glycogen synthetase [18,19]. On the other hand the decrease in the concentration of glycogen in the liver and to a lesser extent that in muscle, the lipolysis of the triglycerides of adipose tissue and liver, osteoporosis and muscular atrophy have long been known as the results of the hypersecretion of the adrenocorticotropin hormone. When all these actions of vasopressin and adrenocorticotropin were taken into consideration together with the actions of the concomitantly released beta-lipotropin and beta-endorphin, which may cause loss of appetite being an opioid, the body weight loss seen in the present study would be a quite natural result.

In the light of the previous and present experimental findings we can reach the following conclusions.

The inhibition of the brain L-asparaginase favours the biosynthesis and/or release of vasopressin and the antidiuretic effect of morphine may be related to its inhibitory effect on the enzyme.

The mechanism which reveals the idiopathic diabetes insipidus can simply be the absolute and/or relative hyperactivity of the brain L-asparaginase. The relative hyperactivity of the brain L-asparaginase depends on the inhibition of asparagine synthetase.

The hyperactivity of the brain L-asparaginase looks the main reason of the impaired development of tolerance to and physical dependence on opiates in homozygous Brattleboro rats.

The single dose of PLG facilitates the development of physical dependence on narcotics by providing the initial inhibition of the brain L-asparaginase which can be easier to be maintained by the further administration of narcotics.

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