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# Arginine-aspartate and haloperidol-induced neurobehavioral effects in the rat

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**Abstract**

This study determined the effects, in the rat, of 8-day treatment with arginine-aspartate on haloperidol-induced catalepsy, decrease of locomotor activity and change of striatal dopamine, homovanillic acid (HVA) and dihydroxy-phenylacetic acid (DOPAC) content. Arginine-aspartate was able to attenuate the haloperidol-induced decrease of locomotor activity and to significantly reduce the catalepsy. Moreover, arginine-aspartate treatment itself increased striatal dopamine content and produced a significant decrease of the HVA/dopamine ratio. Pretreatment with arginine-aspartate was able to partially counteract the haloperidol-induced changes of dopamine metabolism: the haloperidol-induced increases of the DOPAC/dopamine and HVA/dopamine ratios were significantly reduced in arginine-aspartate- pretreated rats. These results suggest that the action of arginine-aspartate on haloperidol-induced neurobehavioral effects is probably mediated by interference with striatal dopaminergic innervation.

*Keywords:* Arginine-aspartate; Catalepsy; Haloperidol; Locomotor activity; Dopamine

## 1. Introduction

Arginine is an amino acid known to produce various effects on the endocrine system, similar to those reported for growth hormone (Ghigo et al., 1994). The introduction of an amino acid into a tissue changes the levels not only of this amino acid, but also of other amino acids in the tissue concerned. Such changes may account for certain pharmacological effects observed following the administration of an arginine salt. Autoradiographic study of the arginine-aspartate tissue distribution, following oral administration in the rat, revealed that the amino acids were widely distributed in various organs, including the brain (Campistron et al., 1982a; Campistron et al., 1982b, 1983). Oral administration of arginine-aspartate stimulated protein phosphorylation in discrete brain regions (Cehovic, 1983). Moreover, chronic treatment with arginine-aspartate was able to reduce the adrenocortical stress response and modify brain free amino acid levels in the rat (Patacchioli et al., 1990).

Unpublished clinical observations showed that arginine-aspartate chronic treatment improved some extrapyramidal symptoms in psychotic patients under long-term treatment with neuroleptics (Paillot et al., 1961). On the basis of these quite old, but very stimulating, observations, we decided to investigate in more depth a potentially new pharmacological effect of arginine-aspartate. For this purpose, we used the experimental model of haloperidol-induced catalepsy and decrease of locomotor activity (Sanberg et al., 1988) as a tool to study the effects of chronic arginine-aspartate treatment on striatal dopaminergic activity in the rat.

Therefore, locomotor activity in 10-min open field sessions, catalepsy and striatal dopamine, homovanillic acid (HVA) and dihydroxy-phenylacetic acid (DOPAC) levels were measured in rats treated with haloperidol (0.5 mg/kg i.p.) after 8-day chronic administration of arginine-aspartate in the drinking water (20 g/l).

## 2. Materials and methods

### 2.1. Animals

The animals were experimentally naive male Wistar rats (250–275 g). Upon receipt from the supplier (Iffa-Credo, Italy), the rats were singly housed in cages and placed in a temperature-regulated (22 ± 2°C) animal room on a 12/12 h light/dark cycle (lights on from 7.00 a.m. to 7.00 p.m.). Food and water were available ad libitum.

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### 2.2. Treatments

After 7 days of rest, animals were randomly assigned to the arginine-aspartate (Asta Medica, Milan, Italy)-treated group or to the control group. Arginine-aspartate was orally administered dissolved in the drinking water (20 g/l). To check the efficacy of the above route of administration, water intake was daily recorded, and the dosage of arginine-aspartate was calculated to be about 5 g/kg body weight per day. Control rats were given only water. Treatment with arginine-aspartate lasted 8 days. No differences in body weight increase were registered between arginine-aspartate-treated and control rats (data not shown). On day 8 after the start of arginine-aspartate treatment, rats were given haloperidol, dissolved in saline (0.5 mg/kg i.p.), or saline injection. Assignment of rats to saline or haloperidol treatment was done randomly. Four experimental groups were examined: Control (drinking water for 8 days and given 0.5 ml of saline i.p. on day 8); AA (drinking arginine-aspartate-enriched water for 8 days and given 0.5 ml of saline i.p. on day 8); HAL (drinking water for 8 days and given 0.5 mg/kg of haloperidol i.p. on day 8); AA + HAL (drinking enriched water for 8 days and given 0.5 mg/kg of haloperidol i.p. on day 8).

### 2.3. Experimental procedures

All behavioral experiments were carried out between 8.00 and 10.00 a.m. in a sound-proof, diffusely illuminated room, maintained at a temperature of 22 ± 2°C.

Spontaneous locomotor activity in an open field was automatically recorded by an Opto-Varimex (Columbus, OH, USA) on day 8 after the start of pretreatment, 2 h after the haloperidol (0.5 mg/kg i.p.) or saline injection. Animals were observed for 10 min in an activity box (40 × 30 cm and 20 cm high) fitted with photocells, and the following items were automatically recorded: ambulation, rearings, non-ambulatory movements, number of starts, number of total movements.

At the end of the locomotor activity session, catalepsy, defined as the acceptance and retention of an abnormal posture, was measured: the forepaws of the rat were placed on a 7 cm high wooden block, and the length of time (s) during which the animal retained this position was recorded.

For neurochemical determinations, animals were killed by decapitation immediately at the end of the catalepsy test. Brains were rapidly removed and the striatum was dissected and kept frozen at −80°C. After tissue homogenization and extraction by $HClO_4$ 0.2 M, containing ethylenediamino-tetraacetic acid (EDTA) 1 mM, $Na_2S_2O_5$ 2 mM and eparine (14 mg/ml), dopamine, HVA, and DOPAC content in the striatum was measured by high-pressure liquid chromatography (HPLC), according to Co et al. (1982). The HPLC system (Kontron Instruments, Italy) utilized a reverse-phase 5 μm μBondapack $C_{18}$ column (3.9 mm × 12.5 cm) coupled to an LC-4B electrochemical detector. The oxidation potential was set at +0.9 V against the reference electrode (Ag/AgCl); the range was set at 20 nA/V. The mobile phase was phosphate buffer 0.04 M, pH 4.3 (containing citric acid 0.06 M, sodium octyl sulfate 2 mM), which was filtered and degassed and methanol was added to a final concentration of 10%. The flow rate was 1.0 ml/min.

Identification and quantification of sample peaks were obtained and calculated by using D450 software (Kontron Instr., Milan, Italy) with reference to the retention times and area under the peak of standards. Results were expressed as pmol/mg protein. Protein measurement was performed according to Lowry et al. (1951).

### 2.4. Statistics

Data, expressed as means ± S.E.M., were statistically evaluated by one-way analysis of variance (ANOVA), followed by the Fisher post-hoc test, as described by Winer (1962).

## 3. Results

### 3.1. Locomotor activity

Table 1 reports the results from the locomotory activity test. When compared to control, 8-day arginine-aspartate

Table 1
Effect of 8-day arginine-aspartate treatment (20 g/l, in drinking water) on haloperidol-induced (0.5 mg/kg i.p.) locomotor activity changes

| Groups | Ambulation | Rearings | Non-ambulatory movements | Starts | Total |
|---|---|---|---|---|---|
| Control ($n$ = 10) | 2384 ± 212 | 79 ± 7 | 3339 ± 237 | 126 ± 9 | 5733 ± 364 |
| AA ($n$ = 8) | 2782 ± 153 | 90 ± 5 | 3441 ± 171 | 127 ± 4 | 6143 ± 290 |
| HAL ($n$ = 10) | 1401 ± 181 [b] | 50 ± 6 [a] | 3338 ± 334 | 82 ± 7 [b] | 4505 ± 380 [a] |
| AA + HAL ($n$ = 10) | 1677 ± 145 [b] | 65 ± 9 | 3627 ± 311 | 77 ± 8 [b] | 5798 ± 430 [c] |
| $F$ (ANOVA) = | 14.25 | 5.47 | 1.16 | 12.49 | 3.00 |
| $P$ = | 0.000 | 0.002 | 0.032 | 0.000 | 0.037 |

Results are expressed as number of movements during a 10-min open field session, and are shown as mean ± S.E.M. In parentheses: number of rats. For group identification, see the Materials and methods section. Statistical significance was determined by ANOVA, followed by Fisher's post-hoc test: [a,b] $P < 0.5$ and 0.01, respectively, vs. control; [c] $P < 0.05$ vs. HAL.

Table 2
Effect of 8-day arginine-aspartate treatment (20 g/l, in drinking water) on haloperidol-induced (0.5 mg/kg i.p., 2 h before test) catalepsy

| Groups | Latency (s) |
|---|---|
| Control ($n = 10$) | 2.3 ± 0.30 |
| AA ($n = 8$) | 2.6 ± 0.32 |
| HAL ($n = 10$) | 16.6 ± 3.37 [b] |
| AA + HAL ($n = 10$) | 5.8 ± 1.10 [a,c] |

Results are shown as means ± S.E.M. In parentheses: number of rats. For group identification, see the Materials and methods section. Statistical significance was determined by ANOVA ($F = 12.77$ $P = 0.000$), followed by Fisher's post-hoc test: [a,b] $P < 0.5$ and 0.01, respectively, vs. control; [c] $P < 0.05$ vs. HAL.

oral treatment produced no statistically significant effects on any of the items examined. Haloperidol, injected i.p. at the dose of 0.5 mg/kg, 2 h before the test, significantly decreased all the parameters, with the exception of the non-ambulatory movements, in comparison to control.

Eight-day pretreatment with arginine-aspartate was able to modify some of the effects of haloperidol on locomotor activity. In fact, the number of total movements of rats pretreated with arginine-aspartate was significantly higher than that of rats treated with haloperidol, remaining at the level of controls. Moreover, ambulation and rearing scores, although not reaching a statically significant level, were higher than those of haloperidol-injected animals. Non-ambulatory movements were not changed in any of the experimental groups.

### 3.2. Catalepsy

As reported in Table 2, oral 8-day treatment with arginine-aspartate did not modify the latency in maintaining the imposed posture in comparison to that of the control group. As expected, 2 h after haloperidol injection (0.5 mg/kg i.p.) rats displayed catalepsy ($P < 0.01$ vs. control). Arginine-aspartate pretreatment significantly attenuated haloperidol-induced catalepsy ($P < 0.05$ vs. haloperidol).

### 3.3. Dopaminergic activity in the striatum

As expected, rats treated with haloperidol had a significant decrease of dopamine content and a parallel increase of HVA and DOPAC. Moreover, the increased DOPAC/dopamine and HVA/dopamine ratios in comparison with controls indicate a significant increase of dopamine metabolism (Table 3).

Chronic arginine-aspartate treatment modified the dopaminergic activity in the striatum. In fact, as reported in Table 3, a 22% increase in dopamine content and a significant decrease of the DOPAC/dopamine ratio was found in comparison with controls. Pretreatment with arginine-aspartate was able to partially counteract the haloperidol-induced increase of dopamine metabolism. In fact, the dopamine content in the AA + HAL group was unchanged in comparison with the control. Moreover, the haloperidol-induced increase of the DOPAC/dopamine ratio was significantly reduced in the arginine-aspartate-pretreated rats, as well as the HVA/dopamine ratio although this did not reach a statistically significant level.

## 4. Discussion

We have presented evidence that chronic treatment with arginine-aspartate produces an inhibition of neuroleptic-induced catalepsy in the rat. In addition, arginine-aspartate was able to partially counteract the effect of haloperidol on locomotor activity. However, arginine-aspartate itself had no apparent influence on locomotor activity or on catalepsy latency.

Experimental catalepsy in the rat is a frequently used procedure to test the effect of a drug on striatal dopaminergic activity (for a review, cf. Sanberg et al., 1988). Haloperidol, a neuroleptic compound, decreases exploratory activity in the rat and produces catalepsy, which has been correlated with a striatal dopamine decrease and HVA increase (Honma and Fukushima, 1976; O'Keeffe et al., 1970). The dose of haloperidol we used to induce catalepsy (0.5 mg/kg i.p.) was that suggested by Ferré et al. (1990) as the most suitable to study the inhibition of neuroleptic-induced catalepsy caused by a dopamine receptor agonist.

Data reported in this paper show that oral treatment for 8 days with arginine-aspartate increased the dopamine content and HVA/dopamine ratio and significantly attenu-

Table 3
Effect of 8-day arginine-aspartate treatment (20 g/l, in drinking water) on haloperidol-induced (0.5 mg/kg i.p.) changes of the dopaminergic system in the striatum

| Groups | Dopamine | DOPAC | HVA | DOPAC/dopamine | HVA/dopamine |
|---|---|---|---|---|---|
| Control ($n = 10$) | 467 ± 41 | 135 ± 8 | 44.7 ± 2.33 | 0.31 ± 0.03 | 0.099 ± 0.006 |
| AA ($n = 8$) | 599 ± 55 [a] | 110 ± 16 | 38.6 ± 4.90 | 0.18 ± 0.01 [b] | 0.064 ± 0.010 |
| HAL($n = 10$) | 375 ± 32 [a] | 197 ± 18 [a] | 105.1 ± 12.6 [b] | 0.57 ± 0.08 [b] | 0.295 ± 0.040 [b] |
| AA + HAL ($n = 10$) | 458 ± 37 | 161 ± 13 [a] | 90.1 ± 6.40 [a] | 0.38 ± 0.05 [c] | 0.217 ± 0.030 [b] |
| $F$ (ANOVA) = | 2.37 | 6.45 | 14.74 | 6.15 | 11.45 |
| $P$ = | 0.082 | 0.001 | 0.000 | 0.0019 | 0.000 |

Results are expressed as pmol/mg p and are shown as means ± S.E.M. In parentheses: number of rats. For group identification, see the Materials and methods section. Statistical significance was determined by ANOVA, followed by Fisher's post-hoc test: [a,b] $P < 0.5$ and 0.01, respectively, vs. control; [c] $P < 0.05$ vs. HAL.

ated the dopaminergic changes induced by haloperidol in the rat striatum, which is a main site of action for the behavioral, biochemical and extrapyramidal effects of haloperidol. Clinically, the extrapyramidal side effects of antipsychotic drugs can be controlled by anticholinergic agents with apparently preserved antipsychotic activity. Catalepsy can be antagonized both by anticholinergic agents and by drugs which increase striatal dopaminergic activity (Andén, 1972).

It appears reasonable to conclude from our study that arginine-aspartate overcomes the haloperidol-induced neurobehavioral effects in the rat by increasing striatal dopaminergic activity, although further experiments are needed to support this hypothesis and to clarify if pretreatment with arginine-aspartate preserves antipsychotic properties of haloperidol.

## References

Andén, N.E., 1972, Dopamine turnover in the corpus striatum and the limbic system after treatment with neuroleptic and anti-acetylcholine drugs, J. Pharm. Pharmacol. 24, 905.

Campistron, G., R. Guiraud, J. Cros and J. Mosser, 1982a, Pharmacokinetics of arginine and aspartic acid administered simultaneously in the rat. I. Plasma kinetics, Eur. J. Drug Metab. Pharmacokin. 7, 307.

Campistron, G., R. Guiraud, J. Cros and G. Prat, 1982b, Pharmacokinetics of arginine and aspartic acid administered simultaneously in the rat. II. Tissue distribution, Eur. J. Drug Metab. Pharmacokin. 7, 315.

Campistron, G., R. Guiraud, J. Cros and H. Pontagnier, 1983, Pharmacokinetics of arginine and aspartic acid administered simultaneously in the rat. III. Changes in the levels of amino acids in the plasma, liver and brain after simultaneous administration of arginine and aspartic acid, Eur. J. Drug Metab. Pharmacokin. 8, 281.

Cehovic, G., 1983, Role des petits peptides sur la phosphorylation des protéines cérébrales in vitro, Acad. Sci. Paris 296, 351.

Co, C., J.E. Smith and J.D. Lane, 1982, Use a single compartment LCEC cell in the determinations of biogenic amine content and turnover, Pharmacol. Biochem. Behav. 16, 641.

Ferré, S., T. Guiz, G. Prat, F. Jane and M. Casas, 1990, Is experimental catalepsy properly measured?, Pharmacol. Biochem. Behav. 35, 753.

Ghigo, E., G.P. Ceda, R. Valcavi, S. Goffi, M. Zini, M. Mucci, G. Valenti, D. Cocchi, E.E. Muller and F. Camanni, 1994, Low doses of either intravenously or orally administered arginine are able to enhance growth hormone response to growth hormone releasing hormone in elderly subjects, J. Endocrinol. Invest. 1994.

Honma, T. and H. Fukushima, 1976, Correlation between catalepsy and dopamine decrease in the rat striatum induced by neuroleptics, Neuropharmacology 15, 601.

Lowry, O.H., N.J. Rosebrough, A. Farr and R.J. Randall, 1951, Protein measurements with the Folin phenol reagent, J. Biol. Chem. 193, 265.

O'Keeffe, R., D.F. Sharman and M. Vogt, 1970, Effect of drugs used in psychoses on cerebral dopamine metabolism, Br. J. Pharmacol. 38, 287.

Paillot M., J. Mouradoff and J.-P. Cagnet, 1961, Traitement de l'asthénie physique et psychique due aux neuroleptiques, Psychol. Méd. 3, 2.

Patacchioli, F.R., M.A. Di Cesare, M.A. Micci and L. Angelucci, 1990, Effect of on free amino acid levels in various brain regions and pituitary of stressed rats, in: Amino Acids. Chemistry, Biology and Medicine, eds. G. Lubec and G.A. Rosenthal (Escom, Leiden) p. 607.

Sanberg, P.R., M. Giordano, M.D. Bunsey and A.B. Norman, 1988, The catalepsy test: its ups and downs, Behav. Neurosci. 102, 748.

Winer, B.J., 1962, Statistical Principles in Experimental Design (McGraw Hill Kogakusha, Tokio).