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## Behavioural and Neurochemical Consequences of Nandrolone Decanoate and Amino Acids Abuse in Rats

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### ABSTRACT

Aggression is a common adverse effect in Anabolic Androgenic Steroids (AASs) abusers. The present study aimed to investigate possible interaction between nandrolone decanoate and amino acids on behavior and neurotransmitters. Rats received repeated injections of nandrolone (10 mg kg<sup>-1</sup>, i.m., once weekly), amino acids mixture (0.8 g kg<sup>-1</sup>, p.o., once daily) and their combination for eight weeks. Defensive aggression, open field and hot plate tests were measured at the end of experiments. Serotonin (5-HT), Dopamine (DA), Norepinephrine (NE) and glutamate were measured in specific brain regions. Analysis of data showed that nandrolone, amino acids and their combination increased aggression and differentially affected brain neurotransmitters. Nandrolone decreased rearing, while amino acids decreased ambulation, increased rearing and grooming frequencies. Taken together, our data indicate that treatment with nandrolone decanoate, amino acids mixture and their combination may induce neurochemical alterations in brain regions regulating aggressive behavior.

**Key words:** Anabolic steroids, amino acids, aggression, neurotransmitters

### INTRODUCTION

Anabolic Androgenic Steroids (AASs) abuse is a prevalent social problem and no longer limited to professional athletes. Adolescents abuse AAS to increase muscular development, improve physical fitness and tolerance to high intensity training (Hartgens and Kuipers, 2004; Talih *et al.*, 2007; Matrisciano *et al.*, 2010). More recently, the negative psychiatric side effects of AASs use have received attention (Lumia and McGinnis, 2010). AASs use has been associated with a propensity for indiscriminate and unprovoked aggression and violence in humans. This gratuitous display of aggression and violence has been referred as "roid rage" (Trenton and Currier, 2005). Today, violence and aggression are the most serious problems facing society (Wood *et al.*, 2012).

There is an increased awareness of the fact that specific nutritional substances are thought to influence physiological functions in the body to improve performance (Maughan and Shirreffs, 2012; Tokaev *et al.*, 2011). Among these, amino acids have proven to be effective in improving performance, reducing fatigue and maintaining a favourable physical condition (Bishop, 2010; Colombani and Mettler, 2011). Indeed, the use of amino acids supplements is gaining popularity in sport community with a commonly held view that they are safe and free of

toxic effects. While several studies reported side effects of amino acids mixtures such as impairment of hepatic and renal function, little is known about possible effects of amino acids mixture on behaviour.

However, less attention has been paid to the effects of stacking combinations of anabolic steroids and amino acids on behaviour and neural circuits that underlie these behavioural effects.

In this context, the present study aimed to assess how AASs and amino acids interaction affect behavioural and physiological responses in rats since amino acids have both direct and indirect effects on neurotransmitters. The studied agents were administered either separately or in combination to accurately reflect human abuse paradigm.

## **EXPERIMENTAL PROCEDURES**

**Animals:** The experiments were carried out using adult male albino rats, weighing 120-140 g. Animals were obtained from the animal house (National Research Centre, Giza, Egypt). All animals were housed under conventional laboratory conditions throughout the period of experimentation and fed standard laboratory pellets (20% proteins, 5% fats, 1% multivitamins) and allowed free access to tap water. Animals were allowed at least one week of acclimatization before using them. Experimental protocols were approved by the Research Ethical Committee of the National Research Centre (Giza, Egypt).

**Drugs:** Nandrolone Decanoate (Nile Pharmaceutical Company, Egypt) was dissolved in arachis oil and injected intramuscularly in a dose of 10 mg kg<sup>-1</sup>, once per week (Kurling *et al.*, 2005). Amino acids mixture (MEPACO Pharmaceutical Company, Egypt) was dissolved in 1% tween 80 in water and used in a dose of 0.8 g kg<sup>-1</sup>, p.o., once daily, 5 days week<sup>-1</sup> (Gibala, 2002).

**Animal treatment:** Rats were randomly assigned to one of six treatment groups; each consisting of 10-12 rats that were treated according to the following scheme: Group 1: received arachis oil, i.m. and served as nandrolone decanoate control, Group 2: received nandrolone decanoate (10 mg kg<sup>-1</sup>, i.m.), Group 3: received 1% tween 80, p.o. and served as amino acids control, Group 4: received amino acids mixture (0.8 g kg<sup>-1</sup>, p.o.), Group 5: received arachis oil and 1% tween 80 and served as combination control and Group 6: received nandrolone decanoate (10 mg kg<sup>-1</sup>), i.m. and amino acids mixture (0.8 g kg<sup>-1</sup>, p.o.). All groups were injected with test agents for 8 weeks.

Basal counts of open field and hot plate tests were recorded at zero time before injection. Behavioural tests were measured at the end of experiments, 24 h after last treatment in the following order: open field, defensive aggression and hot plate tests. All behavioural tests were carried out between 8 am and 1 pm to avoid the effect of diurnal variation.

At the end of experiments, animals were weighed and sacrificed by decapitation 48 h following the last treatment. Brains were rapidly excised, transferred to a dry ice-cold glass plate and dissected into different brain regions (cerebral cortex, striatum, hippocampus and hypothalamus). Brain samples were stored at -80°C till analysis of the following neurotransmitters: 5-HT, DA, NE and glutamate.

### **Behavioral testing**

**Open field test:** Animals were tested in an open field arena using square wooden arena (80×80×40 cm high) divided into 16 equal squares (Pruus *et al.*, 2002). Each rat was placed at the

same corner square and observed during 10 min. Parameters measured were: Ambulation frequency (e.g., number of squares crossed by the animal, rearing frequency (e.g., number of times the animal stood stretched on its hind limbs with or without forelimb support) and grooming frequency (e.g., number of face scratching, washing with the hind limbs and licking of the forelimbs).

**Defensive aggression test:** The rat was lifted by its tail and placed in a plexiglas cage (60×31×41 cm high) and allowed to habituate for 30 sec. The rats' reaction to five different stimuli was then assessed according to Johansson *et al.* (2000).

**The hot plate test:** Each animal was gently placed onto a 52±0.1°C hot plate to perform the test. Latency to exhibit nociceptive responses, such as licking paws or jumping off the hot plate was determined at zero time (pre-treatment) and 24 h after last injection of test drugs or their corresponding controls (Woolfe and McDonald, 1944).

**Neurochemical analyses:** The following endogenous compounds in various brain regions were determined by the use of High Performance Liquid Chromatography (HPLC) according to the method of Pagel *et al.* (2000): NE, DA and 5-HT. Prior to analysis, brains were homogenized in 1/5 wt/v of 75% aqueous methanol to obtain 20% homogenate. Each homogenate was centrifuged at 3000 rpm (4°C) for 10 min. The supernatants were injected onto AQUA column C18 (150x4.6 mm I.D., 5 µm) purchased from Phenomenex, USA. Glutamate concentration was determined by mass spectrometry/electron spray ionization technique (MS/ESI) technique according to Zoppa *et al.* (2006).

**Statistical analysis:** Results were expressed as Means±S.E. Comparisons between means were carried out using one-way ANOVA test followed by Tukey-HSD post hoc analysis. The level of significance was set at p<0.05. Graph pad Prism software (version 5) was used to carry out all statistical tests.

## RESULTS

### Behavioral tests

**Defensive aggression:** Administration of nandrolone decanoate or amino acids mixture increased defensive aggression scores to about 100% as compared to control group. Meanwhile, the combination of nandrolone decanoate and amino acids mixture increased defensive aggression by 126.7% as compared to their respective control (Fig. 1).

### Open field test

**Ambulation frequency:** Administration of nandrolone decanoate did not alter the ambulation frequency, while administration of amino acids mixture markedly depressed the ambulation frequency to about 63% as compared to control (38% vs. 104%, p<0.001). Administration of both nandrolone decanoate and amino acid mixture significantly decreased ambulation frequency to about 60% as compared to control (Fig. 2a).

**Rearing frequency:** Nandrolone decanoate depressed the rearing frequency to nearly 45% of the control value (58.7% vs. 108.2%, p<0.001), while oral administration of amino acids mixture showed

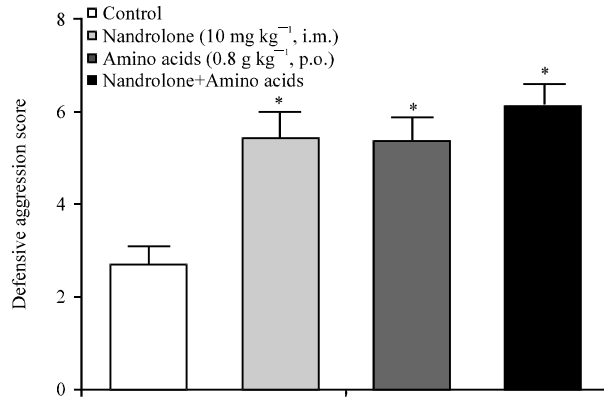


Fig. 1: Effect of 8 weeks treatment with nandrolone decanoate, amino acids and their combination on defensive aggression, Data are Mean±SE, \*Significantly different from corresponding control group at  $p < 0.05$

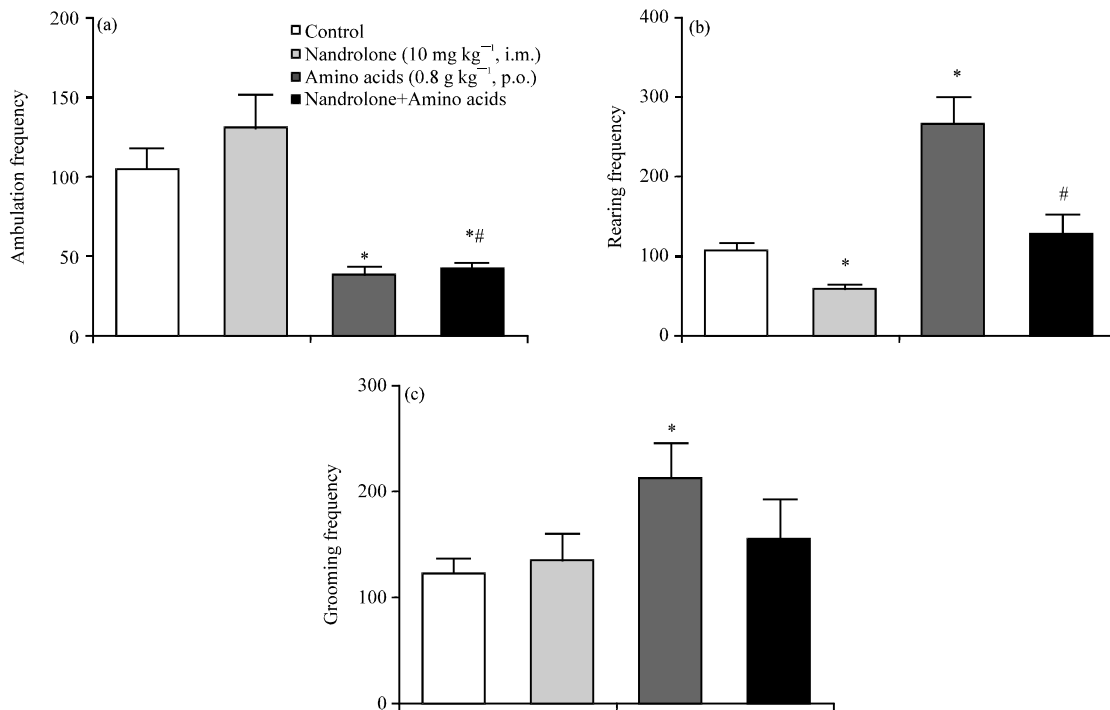


Fig. 2(a-c): Effect of 8 weeks treatment with nandrolone decanoate, amino acids and their combination on, (a) Ambulation, (b) Rearing and (c) Grooming frequencies in open field test, Data are Mean±SE, \*Significantly different from corresponding control group at  $p < 0.05$

marked increase in rearing frequency reaching 146.2% of the control values (265.9% vs. 108.23,  $p < 0.001$ ). Administration of both drugs did not affect rearing frequency as compared to sedentary control group (Fig. 2b).

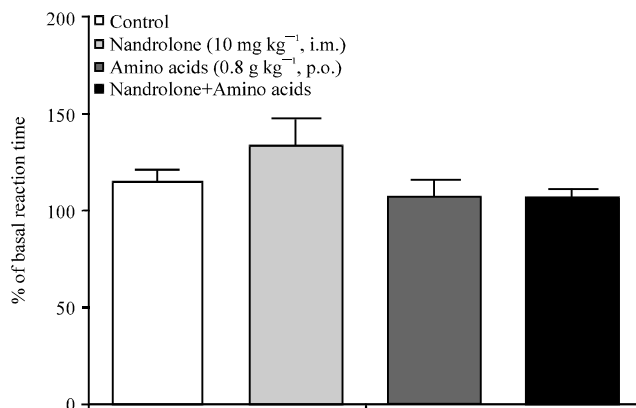


Fig. 3: Effect of 8 weeks treatment with nandrolone decanoate, amino acids and their combination on reaction time in hot plate test, Data are  $\text{Mean} \pm \text{SE}$

**Grooming frequency:** Grooming frequency was not altered in rats receiving nandrolone decanoate at a dose of  $10 \text{ mg kg}^{-1}$  for 8 weeks. On the other hand, rats receiving amino acid mixture exhibited marked elevation in grooming frequency reaching 70% as compared to control group (211.7% vs. 124%,  $p < 0.001$ ). Administration of both nandrolone decanoate and amino acid mixture did not affect grooming frequency (Fig. 2c).

**Hot plate test:** None of the studied drugs at the given doses, significantly altered reaction time in hot plate test as compared to their respective sedentary control group (Fig. 3).

### Neurochemical assays

**Serotonin:** Rats receiving nandrolone decanoate showed decreased serotonin content in striatum (0.6 vs. 9.4,  $p < 0.001$ ) and hippocampus (0.3 vs. 1.5,  $p < 0.01$ ), while no significant change occurred in both cerebral cortex and hypothalamus as compared to control group. Oral administration of amino acids mixture resulted in a decrease in serotonin content in striatum (2.7 vs. 9.4,  $p < 0.001$ ) and hypothalamus (0.04 vs. 0.18,  $p < 0.01$ ). On the other hand, administration of combination of nandrolone decanoate and amino acids significantly decreased serotonin content in all studied regions as compared to the control values (Fig. 4a-d).

**Dopamine:** Treatment with nandrolone decanoate resulted in increased dopamine content in hypothalamus (0.9 vs. 0.4,  $p < 0.01$ ), as compared to control while treatment with amino acids increased dopamine content in hippocampus only (2.2 vs. 1.3,  $p < 0.05$ ). However, a decrease in dopamine content in cortex (9.05 vs. 12.4,  $p < 0.05$ ) and striatum (2.2 vs. 4.04,  $p < 0.01$ ) was found after treatment with combination of nandrolone decanoate and amino acids for 8 weeks as compared to control group (Fig. 5a-d).

**Norepinephrine:** Intramuscular injection of nandrolone decreased norepinephrine contents in hippocampus (0.3 vs. 0.6,  $p < 0.05$ ) only, as compared to control rats. Oral administration of amino acids mixture increased norepinephrine contents in striatum (1.14 vs. 0.65,  $p < 0.01$ ) and

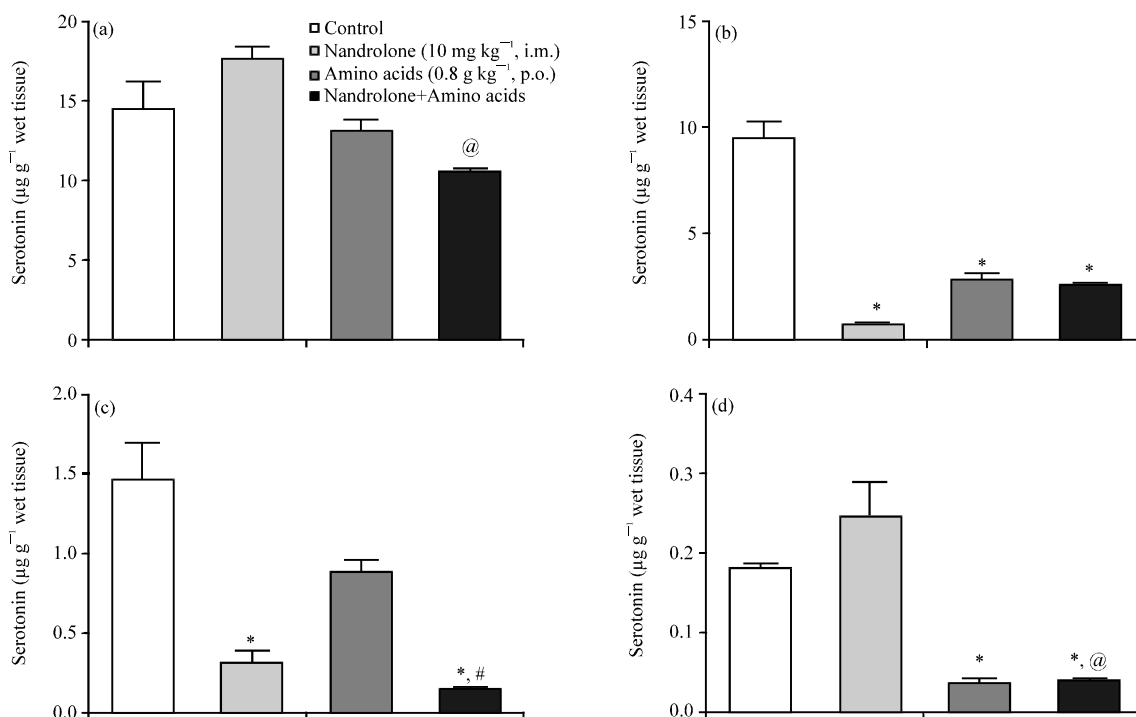


Fig. 4(a-d): Effect of 8 weeks treatment with nandrolone decanoate, amino acids and their combination on serotonin content in, (a) Cerebral cortex, (b) Striatum, (c) Hippocampus and (d) Hypothalamus, Data are Mean±SE, \*Significantly different from corresponding control group at  $p < 0.05$ , @Significantly different from nandrolone-treated group at  $p < 0.05$ , #Significantly different from amino acids-treated group at  $p < 0.05$

hypothalamus (0.34 vs. 0.09,  $p < 0.01$ ), as compared to control. However, combination of both nandrolone and amino acids mixture resulted in a significant reduction in norepinephrine content in hippocampus (0.3 vs. 0.6,  $p < 0.05$ ) as compared to control rats (Fig. 6a-d).

**Glutamate:** Nandrolone decanoate did not alter glutamate content in cerebral cortex, while it increased significantly in hippocampus as compared to control group (50 vs. 25,  $p < 0.05$ ). Oral administration of amino acids mixture did not affect glutamate content in either cortex or hippocampus as compared to control group. Combination of both nandrolone decanoate and amino acids mixture significantly increased glutamate content in cortex (17 vs. 8.7,  $p < 0.01$ ) and hippocampus (64.3 vs. 25,  $p < 0.05$ ) as compared to control group (Fig. 7a, b).

## DISCUSSION

To date, possible effects of combined administration of nandrolone decanoate and amino acids mixture on behavior have never been explored in animal model. The major finding in the present study is that both nandrolone decanoate and amino acids increased defensive aggression scores and their combination further elevated this response. These effects correlated with decrease in 5-HT content in rats receiving either nandrolone decanoate, amino acids or both of them. Moreover, nandrolone affects dopaminergic system. In particular DA content increased in cortex and

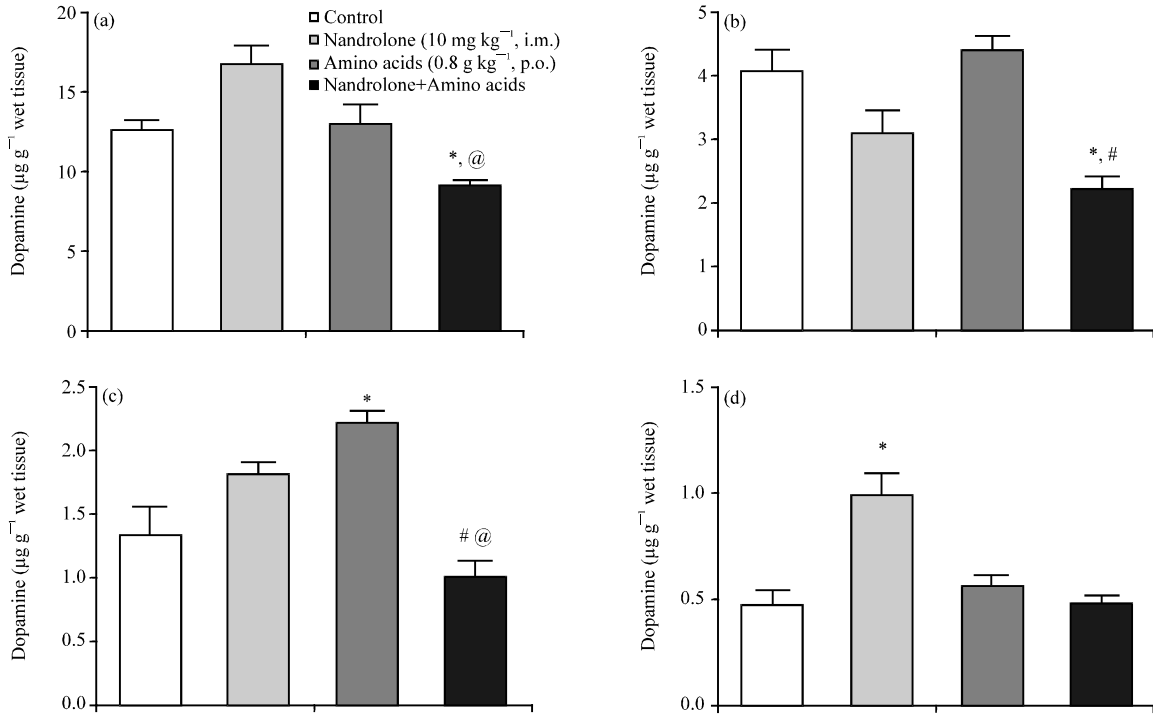


Fig. 5(a-d): Effect of 8 weeks treatment with nandrolone decanoate, amino acids and their combination on dopamine content in, (a) Cerebral cortex, (b) Striatum, (c) Hippocampus and (d) Hypothalamus, Data are Mean±SE, \*Significantly different from corresponding control group at  $p < 0.05$ , @Significantly different from nandrolone-treated group at  $p < 0.05$ , #Significantly different from amino acids-treated group at  $p < 0.05$

hypothalamus. Chronic treatment with supratherapeutic doses of nandrolone decanoate mainly reflects a stimulatory influence on the mesolimbic DA system rather than the nigro-striatal DA system. Stimulation of the mesolimbic DA system is known to be related to reinforcement of behavior so enhanced hyperactivity of the DA system might account for some positive effects as euphoria, increased self-esteem and confidence that frequently appear as early effects following the administration of AASs in humans (Kurling *et al.*, 2005).

Oral administration of amino acids mixture increased defensive aggression. This can be explained by the fact that ingestion of tryptophan (TRP)-free amino acids mixtures in laboratory animals leads to extremely rapid changes in plasma TRP and brain serotonin (5-HT) content with maximal reductions of brain 5-HT occurring within 2 h. It also alters behavioral indices of 5-HT function (increasing pain sensitivity, acoustic startle, motor activity and aggression) (Bell *et al.*, 2001).

Administration of amino acids mixture decreased 5-HT, increased DA in hippocampus only and increased NE in striatum and hypothalamus. The synthesis of neurotransmitters in mammalian brain responds rapidly to changes in precursor availability. 5-HT synthesis depends largely on the brain concentrations of L-TRP, its precursor amino acid. Similarly, the synthesis of catecholamines (e.g., DA and NE) in the brain varies with the availability of the precursor amino acid L-tyrosine (Fernstrom, 1977).



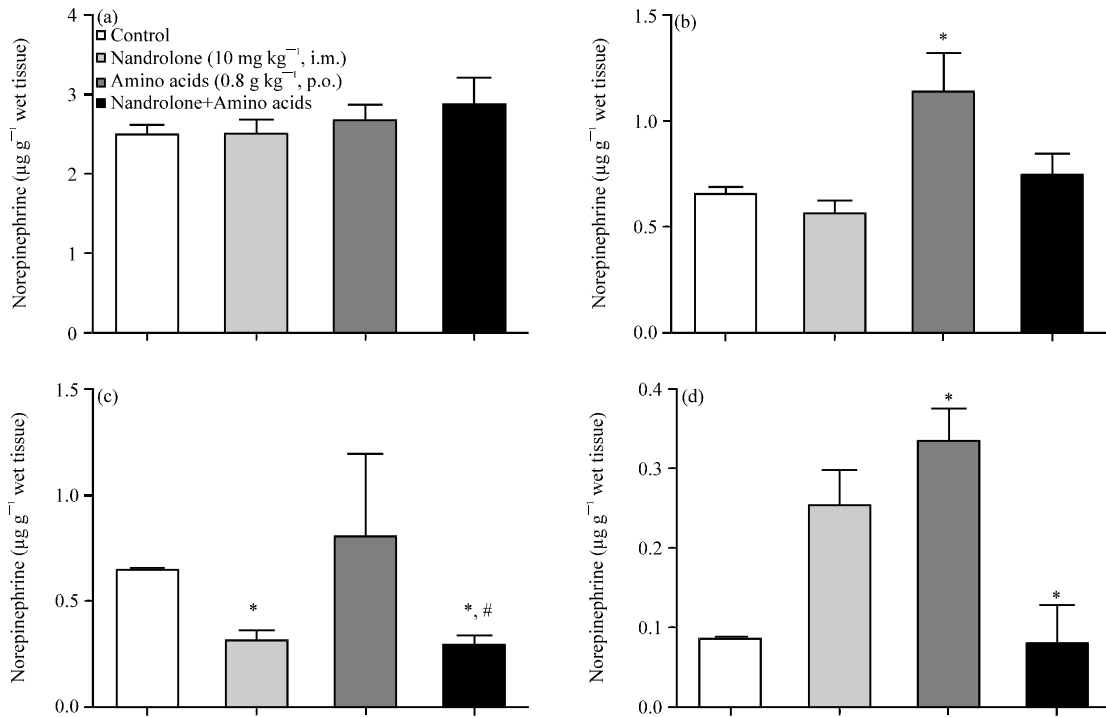


Fig. 6(a-d): Effect of 8 weeks treatment with nandrolone decanoate, amino acids and their combination on norepinephrine content in, (a) Cerebral cortex, (b) Striatum, (c) Hippocampus and (d) Hypothalamus, Data are Mean $\pm$ SE, \*Significantly different from corresponding control group at  $p < 0.05$ , #Significantly different from amino acids-treated group at  $p < 0.05$

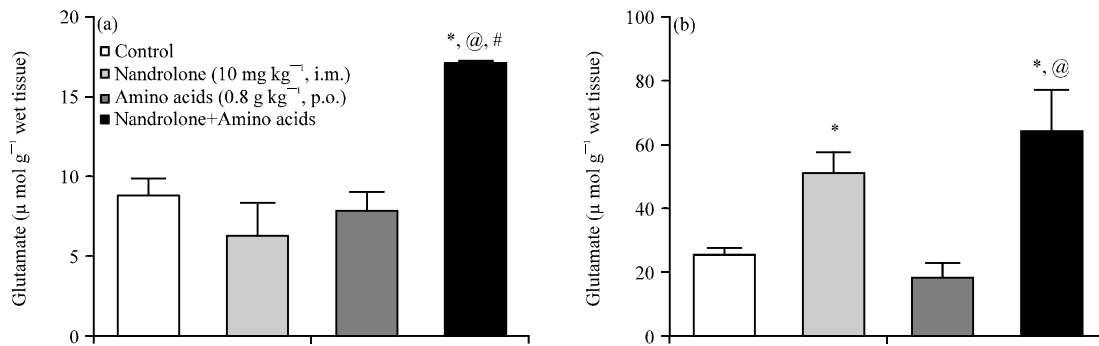


Fig. 7(a-b): Effect of 8 weeks treatment with nandrolone decanoate, amino acids and their combination on glutamate content in, (a) Cerebral cortex and (b) Hippocampus, Data are Mean $\pm$ SE, \*Significantly different from corresponding control group at  $p < 0.05$ , @Significantly different from nandrolone-treated group at  $p < 0.05$ , #Significantly different from amino acids-treated group at  $p < 0.05$

Oral administration of amino acids mixture did not affect glutamate levels in either cortex or hippocampus. The absence of effect on glutamate content suggests that glutamate does not play a key role in amino acids-induced aggression.

Combination of both nandrolone decanoate and amino acids increased aggression. While there exists a large literature on aggression that describes the behavioral and neurochemical sequelae that accompany AASs cocktail administration, no studies have demonstrated that AASs and nutritional supplements administration increases aggressive behavior. In the current investigation, the combination of both nandrolone decanoate and amino acids decreased 5-HT in striatum, hippocampus and hypothalamus, reduced NE content in hippocampus and increased glutamate in cortex and hippocampus. The finding that in nandrolone-amino acids animals there is an increase in glutamate content strengthens the notion that glutamate plays a key role in AAS-amino acid-induced aggression. Since it has been hypothesized that serotonergic neurons play an important role in some types of depressive disorders and also a reduction of noradrenergic transmission is central in depressive disorders, our results suggest that chronic treatment with nandrolone and amino acids seems to reproduce the neurochemical substrate seen in a depressive state.

Open field test was used to gain a general overview of the behavioural characters. Ambulation is an indicator of motor activity. Rearing, on the other hand, is an indicator of exploratory behavior, whereas grooming is considered as a measure of emotional response (Inone *et al.*, 1996; Tamasidze, 2006).

There is a negative correlation between increased levels of 5-HT and locomotor activity (Plaznik *et al.*, 1983). Strombom (1975) proved that norepinephrine is important for exploratory behavior and consequently rearing frequency. Grooming activity was attributed to dopaminergic system (Carneiro *et al.*, 2005).

In the current study, injection of nandrolone decanoate decreased rearing frequency. This was correlated with the observed decrease in norepinephrine content. Amino acids-treated animals showed decreased ambulation frequency, while exhibited marked increase in rearing and grooming frequencies. Decreased locomotion in the open field is viewed as generally indicative of behavioral inhibition (e.g., less freezing). Previous studies have shown that locomotion by the rat in open field is inversely associated with anxiety ratings, depending upon whether the locomotion appears purposeful and whether the animal exhibits other exploratory behaviors such as rearing.

Pain sensitivity in hot plate test was assessed since altered pain sensitivity could contribute to altered responding in aggression testing. Nandrolone decanoate -treated rats did not differ from controls in reaction time in hot plate test. These results are consistent with findings showing that nandrolone decanoate did not affect acute nociceptive thresholds on the hot plate, tail withdrawal and paw pressure tests (Tsutsui *et al.*, 2011).

In the present study, no noticeable effect on reaction time in the hot plate test was observed after oral administration of amino acids mixture. At present, specific studies on possible analgesic effects of amino acids supplements are not available.

In conclusion, the present data indicate that treatment with nandrolone decanoate, amino acids mixture and their combination may induce neurochemical alterations in brain regions regulating aggressive behavior. This finding is of relevant interest considering that often athletes combine many drugs that interfere with 5-HT and DA function.

## **ACKNOWLEDGMENTS**

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## **REFERENCES**

Bell, C., J. Abrams and D. Nutt, 2001. Tryptophan depletion and its implications for psychiatry. *Br. J. Psychiatry*, 178: 399-405.

- Bishop, D., 2010. Dietary supplements and team-sport performance. *Sports Med.*, 40: 995-1017.
- Carneiro, L.M., J.P. Diogenes, S.M. Vasconcelos, G.F. Aragao, E.C. Noronha, P.B. Gomes and G.S. Viana, 2005. Behavioural and neurochemical effects on rat offspring after prenatal exposure to ethanol. *Neurotoxicol. Teratol.*, 27: 585-592.
- Colombani, P.C. and S. Mettler, 2011. Role of dietary proteins in sports. *Int. J. Vitamin Nutr. Res.*, 81: 120-124.
- Fernstrom, J.D., 1977. Effects of the diet on brain neurotransmitters. *Metabolism*, 26: 207-223.
- Gibala, M.J., 2002. Dietary protein amino acid supplements and recovery from exercise. *Sport Sci. Exch.*, 15: 1-9.
- Hartgens, F. and H. Kuipers, 2004. Effects of Androgenic-anabolic steroids in athletes. *J. Sport Med.*, 34: 513-522.
- Inone, I., K. Yanai, D. Kitamura, I. Taniuchi and T. Kabayashi *et al.*, 1996. Impaired locomotor activity and exploratory behavior in mice lacking histamine H1 receptors. *Proc. Natl. Acad. Sci. USA.*, 93: 13316-13320.
- Johansson, P., A.S. Lindqvist, F. Nyberg and C. Fahlke, 2000. Anabolic androgenic steroids affects alcohol intake defensive behaviors and brain opioid peptides in the rat. *Pharmacol. Biochem. Behav.*, 67: 271-280.
- Kurling, S., A. Kankaanpaa, S. Eliermaa, T. Karila and T. Seppala, 2005. The effect of sub-chronic nandrolone decanoate treatment on dopaminergic and serotonergic neuronal systems in the brains of rats. *Brain Res.*, 1044: 67-75.
- Lumia, A.R. and M.Y. McGinnis, 2010. Impact of anabolic androgenic steroids on adolescent males. *Physiol. Behav.*, 100: 199-204.
- Matrisciano, F., A.M.E. Modafferi, G.I. Togna, Y. Barone, G. Pinna, F. Nicoletti and S. Scaccianoce, 2010. Repeated anabolic androgenic steroid treatment causes antidepressant-reversible alterations of the hypothalamic-pituitary-adrenal axis, BDNF levels and behavior. *Neuropharmacology*, 58: 1078-1084.
- Maughan, R.J. and S.M. Shirreffs, 2012. Nutrition for sports performance: Issues and opportunities. *Proc. Nutr. Soc.*, 71: 112-119.
- Pagel, P., J. Blome and H.U. Wolf, 2000. High-performance liquid chromatographic separation and measurement of various biogenic compounds possibly involved in the pathomechanism of Parkinson's disease. *J. Chromatogr. B. Biomed. Sci. Applied*, 746: 297-304.
- Plaznik, A., W. Danysz and W. Kostowski, 1983. Some behavioural effects of microinjections of noradrenaline and serotonin into the hippocampus of the rat. *Physiol. Behav.*, 31: 625-631.
- Pruus, K., A. Vaarmann, R. Rudissaar, L. Allikmets and V. Matto, 2002. Role of 5-HT<sub>1A</sub> receptors in the mediation of acute citalopram effects: A 8-OH-DPAT challenge study. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, 26: 227-232.
- Strombom, U., 1975. Effects of low doses of catecholamine receptor agonists on exploration in mice. *J. Neural. Transm.*, 37: 229-235.
- Talih, F., O. Fattal and D. Jr. Malone, 2007. Anabolic steroid abuse: Psychiatric and physical costs. *Cleve. Clin. J. Med.*, 74: 341-346.
- Tamasidze, A.G., 2006. [Influence of the chronic exposure to network frequency electromagnetic field on rats under interrupted and continuous action of EMF]. *Georgian Med. News*, 140: 91-93.
- Tokaev, E.S., R. Miroedov, E.A. Nekrasov, I.S. Krasnova, A.A. Khasanov and S.M. Ledovskoi, 2011. Influence of specialized protein food products on functional status of wrestlers. *Voprosy Pitaniia*, 80: 83-88.

- Trenton, A.J. and G.W. Currier, 2005. Behavioral manifestations of anabolic steroid use. *CNS Drugs*, 19: 571-595.
- Tsutsui, K.T., R.I. Wood and R.H. Craft, 2011. Anabolic-androgenic steroid effects on nociception and morphine antinociception in male rats. *Pharmacol. Biochem. Behav.*, 99: 500-508.
- Wood, R.I., A. Armstrong, V. Fridkin, V. Shah, A. Najafi and M. Jakowec, 2012. Roid rage in rats? Testosterone effects on aggressive motivation, impulsivity and tyrosine hydroxylase. *Physiol. Behav.*, 110-111: 6-12.
- Woolfe, G. and A.D. MacDonald, 1944. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). *J. Pharmacol. Exp. Ther.*, 80: 300-307.
- Zoppa, M., L. Gallo, F. Zacchello and G. Giordano, 2006. Method for the quantification of underivatized amino acids on dry blood spots from newborn screening by HPLC-ESI-MS/MS. *J. Chromatogr. B*, 831: 267-273.