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The effect of alpha-ketoglutarate (AKG) on the behaviour of ageing laboratory mice

Tomasz Niemiec[1], Klara Zglińska[1#], Wiesław Świderek[2], Weronika Górecka[1], Magdalena Matusiewicz[1], Andrzej Łozicki[1], Iwona Kosieradzka[1], Stefan Pierzynowski[3]

[1]Warsaw University of Life Sciences, Faculty of Animal Sciences,
Department of Animal Nutrition and Biotechnology,
ul. Ciszewskiego 8, 02-786 Warszawa; e-mail: klara_piotrowska@sggw.pl
[2]Warsaw University of Life Sciences, Faculty of Animal Sciences,
Department of Genetics and Animal Breeding,
ul. Ciszewskiego 8, 02-786 Warszawa
[3]Lund University, Department of Cell and Organism Biology,
Sölvegatan 35/37, 223 62 Lund, Sweden

Ageing is a complex physiological process characterized by a gradual decline in cell, tissue and organ function. Consequently, ageing is a major risk factor for cardiovascular disease, diabetes and neurodegenerative disorders. Altered nervous system function can lead to behavioural or psychological disorders. Recent research has shown that alpha-ketoglutarate (AKG) is a key intermediate in the Krebs cycle that extends the lifespan of adult animal organisms. The objective of this study was to assess the effect of AKG on the behaviour of laboratory mice in an open field test. The Na-AKG form was found to enhance perceptual--motor exploration in the mice (expressed as the number of rearings), and at the same time to suppress anxiety-related behaviour.

KEY WORDS: ageing / alpha-ketoglutarate / AKG / behaviour / open field test

Ageing is a complex physiological process characterized by gradual deterioration of organ function. This process progresses over time, making it difficult to maintain homeostasis in the body. In the case of humans, ageing is most often accompanied by pathological changes; in addition to changes in organs resulting from the passage of years, co-morbidities appear as well. The most serious diseases associated with ageing include neurodegenerative diseases, which often make it impossible for elderly people to function independently. The changes that take place in the nervous system of older people (even when the process is not pathological) include slower conduction of nerve impulses in the nervous system, a reduced response to β-adrenergic stimulation, and increased blo-

od vessel stiffness, including in the brain [2, 11]. In ageing animals, dysfunction of the antioxidant defence system has been demonstrated, accompanied by increased generation of reactive oxygen species within the mitochondria and of lipid peroxidation products, as a result of disturbed redox homeostasis [11]. Numerous studies also indicate that reactive oxygen species contribute to the development of many neurodegenerative diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, multiple sclerosis, Huntington's disease, and others [3].

For years, researchers have been searching for biologically active compounds that can prevent cellular ageing processes, preferably by mitigating their causes, particularly the production of excessive amounts of oxidants, and by counteracting the effects of their activity. Alpha-ketoglutarate (AKG) is a molecule that determines the rate of conversions in the tricarboxylic acid cycle in the body. This well-known amine group scavenger stimulates protein synthesis while inhibiting protein degradation in the muscles. AKG, as a source of glutamate and glutamine, is the most important 'fuel' for gastrointestinal epithelial cells [12]. AKG also plays an important role as an antioxidant, taking part in the non-enzymatic inactivation of hydrogen peroxide. Recent studies have shown that AKG can also extend the lifespan of ageing laboratory animals, inhibiting degenerative processes, including those taking place in the nervous system [1, 6, 7].

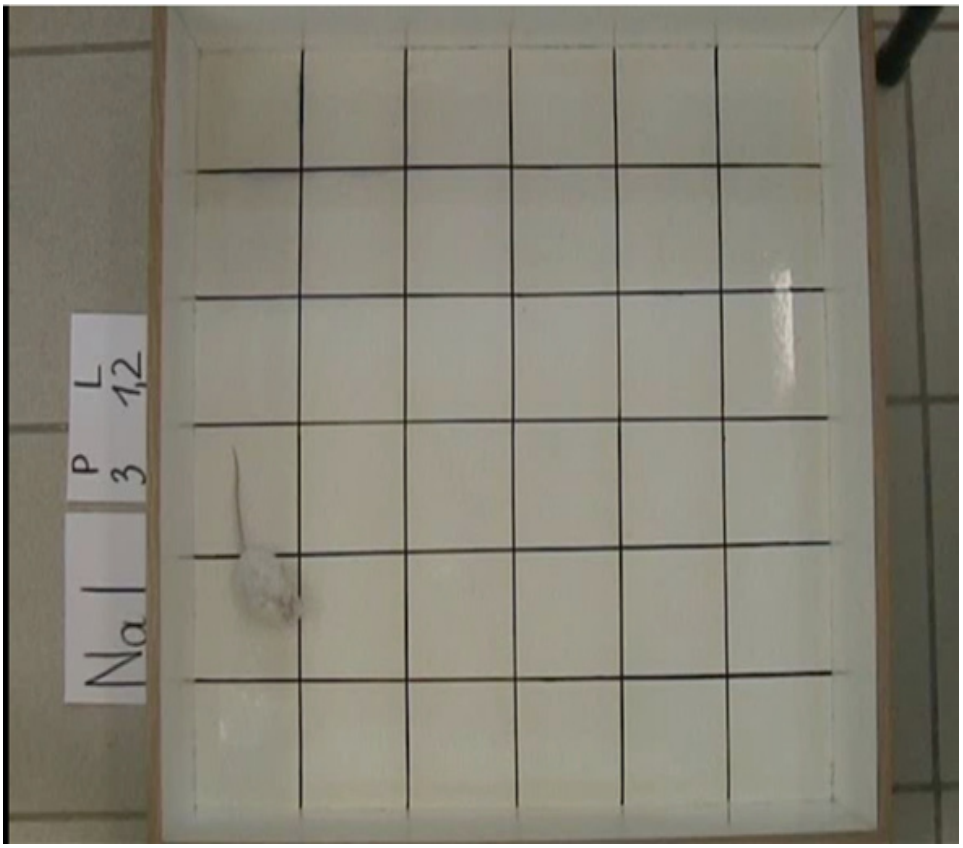
A study was conducted to assess the effect of AKG added to feed on the behaviour of ageing mice in an open field test.

Material and methods

The experiment was carried out on laboratory mice from the Department of Genetics and Animal Breeding, Warsaw University of Life Sciences. Male mice at the age of 12 months were selected for the experiment. The mice were kept in standard conditions, in a 12:12 h dark-light cycle at 22°C and 60% humidity. The animals in the control group (K) were fed standard feed ad libitum (Labofeed H Kcynia, Poland). Two experimental groups were distinguished – P1 and P2, whose feed contained 2% AKG calcium salt (Ca-AKG) and 2% AKG sodium salt (Na-AKG), respectively. The share of the supplement was based on the results of research on pigs and rats [5, 8]. At the end of the experiment, there were eight mice each in the control (K) and the P2 groups, and ten in the P1 group. Some of the animals died from natural causes (old age) during the experiment, which was confirmed by a necropsy performed by a veterinarian.

An 'open field' test was used to perform a behavioural evaluation of the animals. This is a qualitative and quantitative zoopsychological test used to measure motor activity as well as the behaviour and habits of animals. The open field test is useful for determining behavioural and locomotor changes, e.g. under the influence of specific chemical substances. It is based on the natural behaviour of rodents resulting from their need for exploration [10].

The test field was 120 cm x 120 cm and was divided into 36 squares (photo). During the observations, the illuminance of the light source was 700 lm/m^2. To minimize stress, the animals were transferred to the room where the study was carried out 30 minutes before it began.



Phot. Mouse during 'open field' test

Each animal was observed during the test for 5 minutes. Six replications were used. The following parameters were determined:

– number of squares crossed in each minute of the experiment

– number of rearings (associated with the animal's activity and need for exploration, accompanied by increased sniffing) in each minute of the experiment

– number of grooming acts (grooming behaviour as an indicator of stress and emotional tension, an attempt to release emotions) in each minute of the experiment

Locomotor activity was measured on the basis of the number of squares crossed, and exploratory and perceptual activity based on the number of rearings. The level of stress was determined by the number of times the mouse entered the central squares and the time spent in this part of the box.

The results were statistically analysed by calculating basic descriptive statistics in Statistica 1 software. The significance of differences between groups was determined by Kruskal–Wallis ANOVA.

Results and discussion

The characteristics of the locomotor activity of the mice are presented in Figure 1 and Table 1. No significant differences were found in the number of squares crossed by mice in different groups. Mice from group P2, receiving 2% AKG sodium salt in their feed (Me = 140), showed a tendency towards greater locomotor activity in the open field test than the mice from groups P1 (Me = 120) and K (Me = 122).

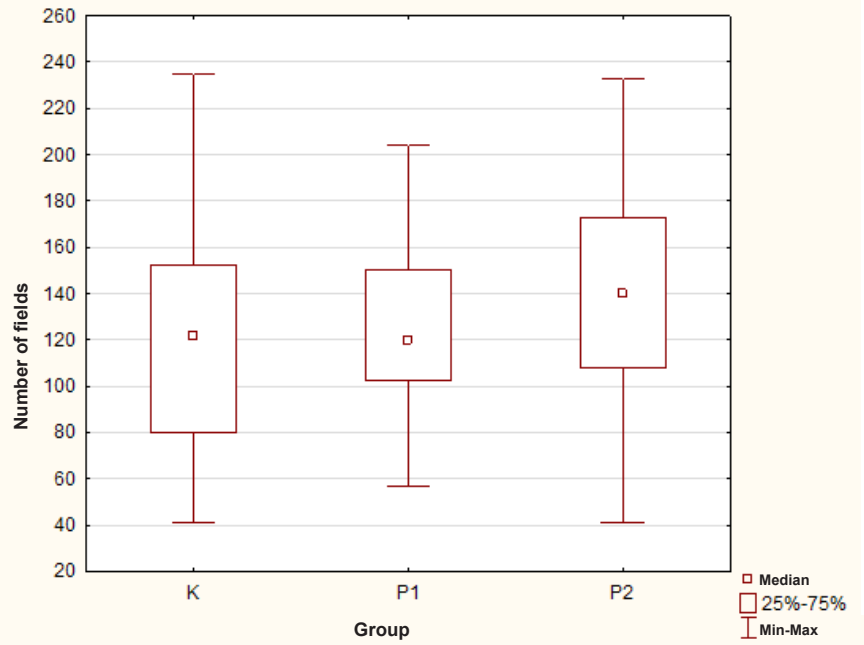


Fig. 1. Variation in locomotor activity of mice in the test groups (K, P1, P2) expressed as number of fields crossed in the ‘open field’ test

Table 1

Basic descriptive statistics of the variable expressed as the number of fields crossed by mice in the 'open field' test

Group	Median (Me)	Lower quartile (Q_1)	Upper quartile (Q_3)	Minimum	Maximum	Coefficient of variation (VQ, %)
K	122.0	80.0	152.5	41.0	235.0	29.7
P1	120.0	102.5	150.5	57.0	204.0	20.0
P2	140.0	108.0	173.0	41.0	233.0	23.2

The number of rearings by P2 mice, which received 2% AKG sodium salt in their feed (Me = 52), was significantly higher than in groups P1 (Me = 33.5) and K (Me = 20). It should also be emphasized that the mice from the control group (K), with lower exploratory activity (number of rearings), showed greater variation in movement expressed by the coefficient of variation (VQ = 83.7%) than the mice in experimental groups P1 (VQ = 40.3%) and P2 (VQ = 19.2%) – Table 2. The data presented show that AKG sodium salt (group P2) had a stronger effect than AKG calcium salt (group P1) on the behaviour of mice (expressed as the number of rearings). The increased perceptual and motor exploration in group P2 indicates that the Na-AKG supplement alleviates the natural fear of a new, potentially threatening environment. In other research on mice of this line [7], AKG has been shown to reduce the effects of oxidative stress by inhibiting lipid peroxidation. Na-AKG reduced the liver concentration of TBARS, a lipid oxidation indicator, more than did Ca-AKG. On the other hand, Ca-AKG caused a decrease in the plasma TBARS level and an increase in total antioxidant status (TAS) [7]. It is likely that the addition of AKG to the diet reduces oxidation of macromolecules in nerve cells, slowing down the neurodegenerative processes associated with ageing, and through this mechanism contributes to greater exploratory activity.

The effect of AKG is not limited to antioxidant activity. The latest research indicates that α-ketoglutarate regulates the cellular metabolism rate by inhibiting the activity of ATP synthase and TOR kinase, which clearly delays ageing processes in nematodes *Caenorhabditis elegans* [1, 12]. According to Satpute et al. [9], oral administration of AKG to mice markedly improved their motor coordination, which confirms the influence of AKG on the nervous system and animal behaviour. It therefore cannot be ruled out that AKG may restrict the progressive ageing of the nervous system by inhibiting the TOR pathway in nerve cells, protecting the brain's lipid domain against the oxidative action of reactive oxygen species (ROS), stimulating the synthesis of individual neuromediators, or improving the vascularization of brain structures responsible for motor coordination in animals.

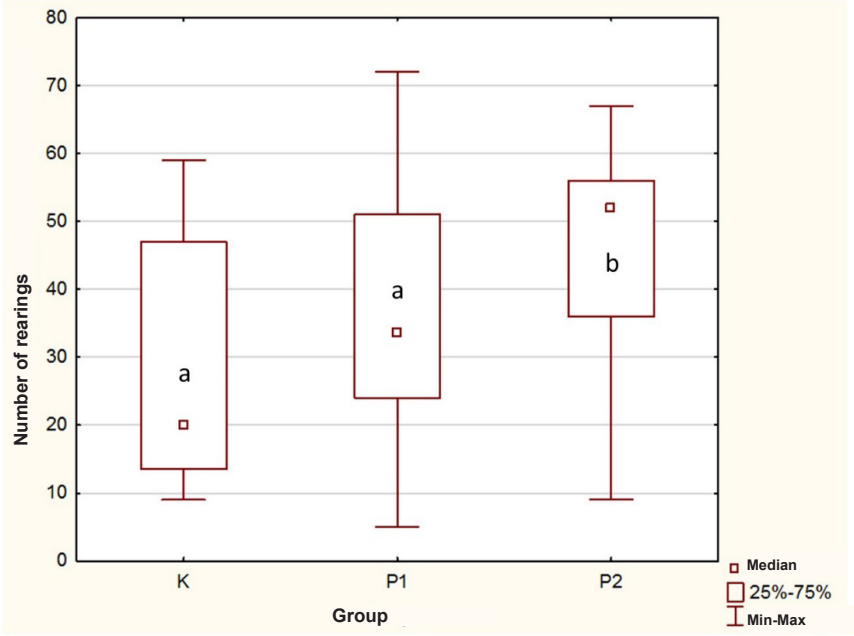


Fig. 2. Variation in exploratory activity of mice in the test groups (K, P1, P2) expressed as the number of rearings in the 'open field' test (for a and b differences significant at $p<0.05$)

Table 2
Basic descriptive statistics of the variable expressed as the number of rearings in the 'open field' test

Group	Median (Me)	Lower quartile (Q_1)	Upper quartile (Q_3)	Minimum	Maximum	Coefficient of variation (VQ, %)
K	20.0[a]	13.5	47.0	9.0	59.0	83.7
P1	33.5[a]	24.0	51.0	5.0	72.0	40.3
P2	52.0[b]	36.0	56.0	9.0	67.0	19.2

a, b – significant differences at $p<0.05$

There were no significant differences in the number of grooming acts performed by mice (Figure 3, Table 3). Animals from the P1 group, however, showed a tendency to

groom more frequently. Grooming behaviour in rodents is a complex pattern of behaviour associated with stress. It is more frequent in animals with autism spectrum disorder (ASD) and obsessive-compulsive disorder (OCD) [4]. The tendency for increased grooming in animals receiving Ca-AKG (group P1) relative to the control group (K) may indicate that

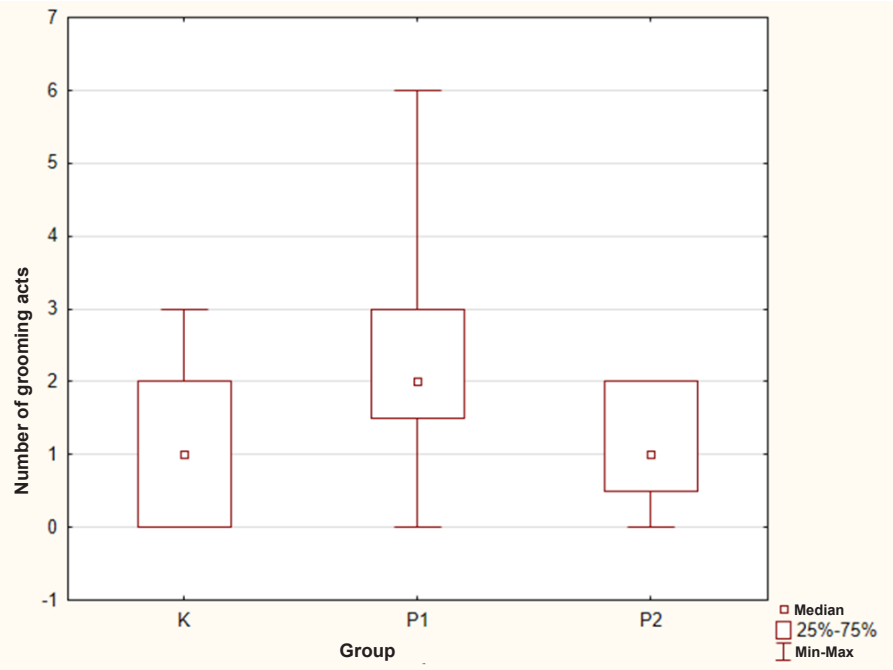


Fig. 3. Variation in grooming behaviours in the test groups (K, P1, P2) expressed as the number of grooming acts in the 'open field' test

Table 3
Basic descriptive statistics of the variable expressed as the number of grooming acts in the 'open field' test

Group	Median (Me)	Lower quartile (Q_1)	Upper quartile (Q_3)	Minimum	Maximum	Coefficient of variation (VQ, %)
K	1.0	0.0	2.0	0.0	3.0	100.0
P1	2.0	1.5	3.0	0.0	6.0	37.5
P2	1.0	0.5	2.0	0.0	2.0	75.0

this form of AKG in mice increases changes associated with behavioural disorders. As the differences in the results were not significant, it is not possible to draw definitive conclusions. In addition, the observations require additional, more detailed tests and analyses.

In summary, the results indicate that AKG administered *per os* in the amount of 2% of feed, both in the form of calcium salt (Ca-AKG) and especially sodium salt (Na-AKG), influenced the behaviour of ageing laboratory mice. The sodium form of AKG appears to have a stronger effect, enhancing perceptual and motor exploration while at the same time suppressing anxiety-related behaviour. The research on the potential of AKG can be of importance not only for animals but for people as well, offering a chance to improve the quality of life of the elderly through measures protecting the nervous system and facilitating the motor functions of the entire body.

REFERENCES

1. CHIN R., FU X., PAI M., VERGNES L., HWANG H., 2014 – The metabolite α-ketoglutarate extends lifespan by inhibiting ATP synthase and TOR. ***Nature*** 510 (7505), 397-401.
2. FIRLAG M., OSTASZEWSKI A., BALASINSKA B., 2014 – Mechanizmy starzenia się ośrodkowego układu nerwowego u zwierząt. ***Życie Weterynaryjne*** 89 (01), 40-45.
3. GUTOWICZ M., 2011 – The influence of reactive oxygen species on the central nervous system. ***Postepy Higieny Medycyny Doświadczalnej*** 18 (65), 104-113.
4. KALUEFF A.V., STEWART A.M., SONG C., BERRIDGE K.C., GRAYBIEL A.M., FENTRESS J.C., 2016 – Neurobiology of rodent self-grooming and its value for translational neuroscience. ***Nature Reviews Neuroscience*** 17 (1), 45-45.
5. KRISTENSEN N.B., JUNGVID H., FERNÁNDEZ J.A., PIERZYNOWSKI S.G., 2002 – Absorption and metabolism of alpha-ketoglutarate in growing pigs. ***Journal of Animal Physiology and Animal Nutrition*** 86 (7-8), 239-245.
6. MCLAIN A.L., SZWEDA P.A., SZWEDA L.I., 2011 – α-Ketoglutarate dehydrogenase: A mitochondrial redox sensor. ***Free Radical Research*** 45 (1), 29-36.
7. NIEMIEC T., SIKORSKA J., HARRISON A., SZMIDT M., SAWOSZ E., 2011 – Alpha-ketoglutarate stabilizes redox homeostasis and improves arterial elasticity in aged mice. ***Journal of Physiology and Pharmacology*** 62 (1), 37-43.
8. PIERZYNOWSKI S.G., FILIP R., HARRISON A., 2007 – Effect of feed supplementation with α-ketoglutarate, combined with vitamin B6 or C, on the performance and haemoglobin and amino acid levels in growing rats. ***Bulletin of the Veterinary Institute in Pulawy*** 51, 289-296.
9. SATPUTE R., LOMASH V., KAUSHAL M., BHATTACHARYA R., 2013 – Neuroprotective effects of α-ketoglutarate and ethyl pyruvate against motor dysfunction and oxidative changes caused by repeated 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine exposure in mice. ***Human & Experimental Toxicology*** 32 (7), 747-758.
10. TROJAN M., MATYSIAK J., 2007 – Próba standaryzacji testu otwartego pola. [W:] Zachowanie się zwierząt. Przegląd wybranych zagadnień z zakresu psychologii porównawczej. VIZJA PRESS&IT, Warszawa, ss. 30-70.

11. WIECZOROWSKA-TOBIS K., 2008 – Organ alterations due to aging. ***Polskie Archiwum Medycyny Wewnętrznej*** 1 (18), 63-69.
12. WU N., YANG M., GAUR U., XU H., YAO Y., LI D., 2016 – Alpha-Ketoglutarate: Physiological Functions and Applications. ***Biomolecules & Therapeutics*** 24 (1), 1-8.