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Effect of *Panax ginseng* Extract on Passive Avoidance Retention in Old Rats

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Abstract □ Female rats of two groups (6 and 27 months) were tested in the passive avoidance test to investigate the age-dependency of the learning ability. The results showed a significantly better avoidance behavior in the young adult animals compared to the older ones. The influence of a 13-day treatment with *Panax ginseng* (30 mg/kg/d, oral) on 27 month old rats caused a considerably prolonging of the latency time in comparison to the untreated control group of the same age. In the open field the treated rats exhibited neither an altered locomotion nor exploration nor a changed emotional reactivity which could explain the improved avoidance reaction.

Key words □ Passive avoidance, open field, *Panax ginseng*.

The influence of age on the learning ability or memory has been tested in numerous experiments with very different test methods. These have proven that there is a remarkably wide range of learning disabilities in age by both humans and animals[1,2]. A limited acquisition of new memories, reduced ability to modify known behavioral reactions, disturbed relearning and inferior short and long-term memory are the most noticeable deficits.

Above all in the passive avoidance test a poorer test result has been observed with increasing age in rats and mice *in the cross-sectional analysis*[3-5]. On the basis of a negative experience, old rats are apparently much less able than young rats to adequately modify their behavior to the same situation 24 h later. It is more likely that an insufficient memory is responsible for this rather than a lack of acquisition. An age-related reduction of the sensitivity to the electric foot stimulus which the animal receives cannot be held responsible for the poorer test results according to Lippa *et al.*[6] and Dean *et al*[4]. Numerous investigators have attributed the incorrect behavior of the older rats to avoid, by means of passive perseverance, the unpleasant stimuli to an age-related limited ability to inhibit behavioral intentions[7,8]. In the past there have been many attempts made to improve the learning disability of the elder rats with various phamaceuticals[9,10]. Furthermore, the extent to which the results of these experiments offer insights into the neuronal mechanisms which participate in the learning process and are responsible for a successful reaction has often been tested. Above all, the cholinergic transmitter system (hippocampus, limbic system) is of interest, because it is apparently essential for the functional integration of learning processes[11]. The modulation of neuronal processes by peptides, adrenergic and serotonergic transmitters in the learning process is controversial[12,15].

In this experiment we tested to what extent *Panax ginseng* influences the learning deficit of old rats. Ginseng has been employed for thousands of years, particularly in Asian folk medicine, as a tonic for restitution or "adaptogen"[16]. Although a number of ginsenosides (glycosides with saponin character) have been isolated from the mixture preparation[17], a direct substance-specific effect *in vivo* in humans and animals has not yet been satisfactorily proven. Among other things a stimulation of the metabolism, stabilization of blood pressure or blood sugar after stress and increased contractile strength of the skeletal muscle during a physical exercise have been

described[18-20]. Many investigators assume that the effect of ginseng can be attributed to an influence of hormonal mechanisms, because stimulating effects on the excretion of adreno-corticotropic and estrogenic hormones has been proven[21]. These results concur with those which have shown an equalizing effect in stress situations[22]. Only a few animal experimental studies were suited to unequivocally demonstrate the physiological and psychomotoric effect of *Panax ginseng*. Kim[23] treated mice and determined that the animals reacted with increased glucocorticoid secretion rates in the cold stress. Other studies have shown that besides stimulating effects ginseng also has a sedative effect[24,25]. The doses used is important because a bell-shaped doses-effect ratio has been described for ginseng[22,26] observed poorer performance in avoidance of the foot shock in the active avoidance test and attributed this effect to a reduced motor function of the rats. Petkov and Mosharrof[22] obtained good test results in the active avoidance test with mice using 10 mg/kg. In operant conditioning tasks 30 mg/kg was effective and 100 mg/kg increased the locomotor activity drastically.

Two hypotheses are to be tested in the following study:

1) 27 month old rats are less successful in learning the passive avoidance test than 6 month old rats.

2) Treatment with *Panax ginseng* for 13 days improves the learning ability of 27 month old rats. The effect is not related to changed locomotion or exploratory activity.

METHOD

Female Wistar rats aged 6 and 27 months (breeder Hagemann, Bosingfeld/Lippe) were tested. The rats were individually housed in Macrolon cages III with a light/dark cycle of 12 h and fed lab chow (Altromin 1320) ad lib. 10 old animals received red *Panax ginseng* extract (*Panax ginseng* C.A. Meyer radix rubra, 30 mg/kg/d) dissolved in their drinking water for 13 days. Ginseng was donated by the Korea Ginseng & Tobacco Research Institute, Taejeon, Korea. Tap water was offered to the controls of the same age ($n=10$) and the young rats ($n=10$). The body weight of rats, as well as their food and fluid consumption were determined daily.

On four consecutive days from the 7th day of treatment rats were placed in the bright part of the test box (50×22×22 cm). The latency they crossed to the black compartment of the box and *vice versa* in two minutes was counted. On the 4th day, the rats received an unavoidable foot shock (2.5 mA, 3 sec) immediately after they had entered the black part. Then they were placed back in their home cage. Whether the rats remembered the shock and avoided reentering the dark was examined 24 h later. The maximum time of 2 min was assigned as avoidance reaction. On the following 2 days the re-tests were repeated.

Differences between treated rats and controls were statistically evaluated by means of analysis of variance. The critical level of significance was set at 5%.

To test to what extent the behavior of untreated rats in an unfamiliar environment is related to age and whether it is influenced by *Panax ginseng*, the rats were observed in an open field test at the end of the treatment period on the 13th day (6 versus 27 month old rats). The test animals were individually placed in a 1 m^2 area made of black wood whose floor was divided into 16 squares. The field was surrounded by 30 cm high walls. The test was performed in red light in accordance with the natural activity rhythm of the rats following the daily light change. The test animal was placed in the area with the head pointed to a corner and observed for 5 min. The number of crossings (crossing into a different section with all four extremities), rearings, groomings and the frequency of defecation (number of defecation boli) were recorded.

The measured values were checked for differences by means of Student's t-test. The level of significance was 5%.

RESULTS

Deviations from the controls were not observed in either body weight or food intake during the daily intake of *Panax ginseng* by the treated rats. Neither were there any withdrawal symptoms after the discontinuation of the substance (Fig. 1).

The results of the passive avoidance test showed that the latency of the change from the light to the dark compartment dropped quickly during the 4-day preliminary phase in the 6 and 27 month old rats independently of the treatment. This can

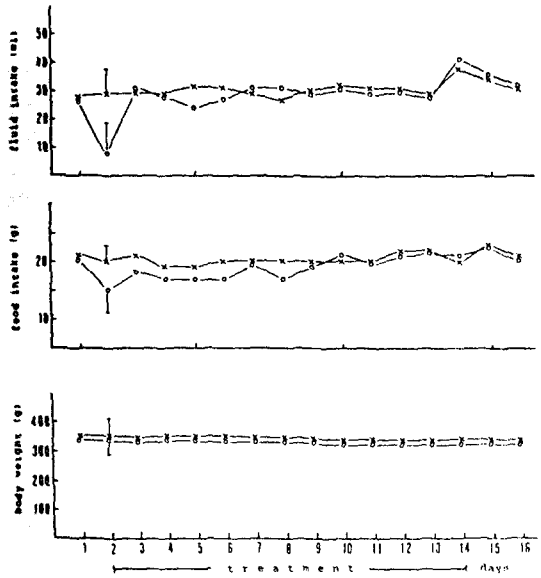

Fig. 1. Fluid and food intake and body weight of 27 month old rats under the influence of *Panax ginseng* (30 mg/kg/d) taken orally via drinking water compared to controls.
x± SD, n=10 per group. O=treated rats, x=controls.

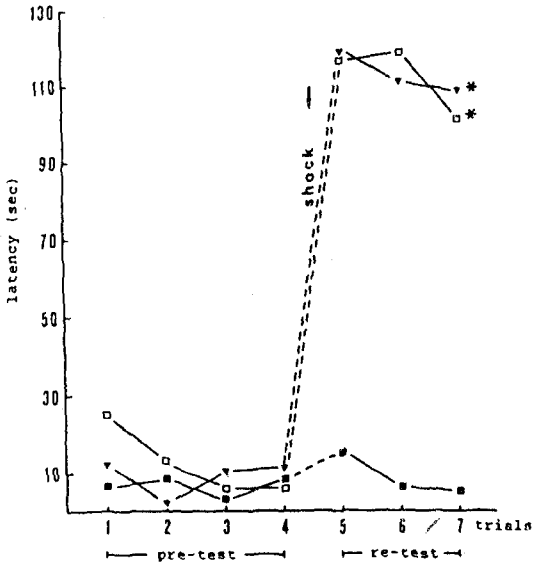

Fig. 2. Behavior of rats of different ages in the passive avoidance test and the influence of *Panax ginseng* on learning ability in old rats.
Mean values, n=10 per group. ▼=months, ■=27 months, □=27 months treated rats.

be described as an adaptation factor (Fig. 2). Almost all of the young rats avoided the dark compartment in the re-test 24 h after the foot shock. The latency to entering the dark chamber was 120± 10 sec. (X± SEM) on average. In contrast, 27 month old rats, which had received water, entered the compartment after a short time. The animals which received *Panax ginseng* on the other hand behaved significantly different ($p<0.05$). Thier latency time did not differ from that of the younger untreated rats, i.e. the animals remained in the light chamber considerably longer than the controls of the same age. This behavior remained the same on the three following days of the re-test phase.

An age related reduction in the locomotion and exploration activity between the 6 and 27 month old untreated rats could be statistically proven ($p<0.05$) in the open field test. Defecation and grooming were the same. Treated and untreated 27 month old animals, however, did not differ in either exploration, locomotion, emotional reactivity (defecation) or frequency of grooming.

DISCUSSION

The results of this study have made it clear that the 13 day treatment with *Panax ginseng* positively influences the cognitive performance of 27 month old rats in the passive avoidance test. An explanation of the deviant behavior on the basis of an altered arousal level can be excluded, because the animals did not exhibit shorter or longer latency values until entering the dark compartment in preliminary phase of the test. Furthermore, the unchanged behavior in the open field test shows that *Panax ginseng* causes neither a stimulating nor sedating effect in the doses employed. This result is important in that it is known that the aging process is often accompanied by a change of the reaction to exogenous and endogenous stimuli (Pharmaceuticals, physical stimuli). On the basis of the disinhibition hypothesis[27] in accordance to which the behavior reactions of old individuals often become incorrect by insufficient inhibition, the effect of *Panax ginseng* can be interpreted as the equalizing effect of an inhibition deficit. What biochemical correlates are of importance for the behavior observed

in the rats treated with *Panax ginseng* must be investigated in further studies. Metabolic effects are not suspected of being important for the behavior observed.

It is conceivable that the attentiveness of the treated rats was increased during the test period. This would lead to a more efficient processing of the stimuli and in conjunction with an undisturbed memory, to more successful behavior. It should be possible by employing other behavioral tests and combining these with results from neurophysiology to strengthen the proof of an increase in vigilance.

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