species derived from oxygen[9], namely the superoxide radical ($O_{2\cdot}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\cdot$ OH). The latter species has been termed 'the most reactive oxidizing species known'.

It has been known for several years that $\cdot$ OH was a reaction product in several in vitro biochemical systems in which H_2O_2 and $O_{2\cdot}$ were present simultaneously[10]. In these studies, it was shown that both catalase, which decomposes H_2O_2, and superoxide dismutase, which decomposes $O_{2\cdot}$, could each by themselves prevent $\cdot$ OH formation. It was quite logically suggested[10] that the $\cdot$ OH was formed in a reaction between H_2O_2 and $O_{2\cdot}$. However, it has been demonstrated by several investigators using various techniques, that the direct reaction between H_2O_2 and $O_{2\cdot}$ is extremely slow[11-14]. Despite these apparent discrepancies a reaction sequence which can account for all of this seemingly inconsistent data can be written as follows[4-7]:

a) $Fe^3 + O_{2\cdot} \rightarrow Fe^{2+} + O_2$

b) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$

c) $O_{2\cdot} + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$

Reaction c, the sum of reactions a and b, is commonly referred to as the Haber-Weiss reaction[15]. It has been suggested[4-7] that the iron-chelator DETAPAC could prevent $\cdot$ OH formation, probably by inhibiting reaction a or b above. It was also determined that EDTA, could promote the rate of $\cdot$ OH formation. In other studies, it has been shown that DETAPAC and other iron-chelating agents could prevent $\cdot$ OH formation only at certain iron-chelator ratios. And in fact, stimulation of $\cdot$ OH formation has been observed at certain ratios of iron to DETAPAC[16].

In recent in vitro experiments with isolated islet preparations, it has been shown that DETAPAC could counteract the toxic actions of alloxan[2,3]. In these same studies, catalase, superoxide dismutase and several potent $\cdot$ OH scavengers were also found to protect against alloxan. All of these data collectively seemed to suggest that $\cdot$ OH, generated from alloxan after its reduction to dialuric acid and further autoxidation, could damage beta cells in vitro.

The data of the present study, taken together with our previous data on protection against alloxan in vivo by several structurally diverse $\cdot$ OH scavengers[17-22], would appear to be consistent with the premise that DETAPAC effectively prevents $\cdot$ OH formation in vivo under the present experimental conditions and that $\cdot$ OH is responsible for alloxan-induced diabetes. Other mechanisms of protection, most likely involving iron, are also possible.

1 Supported in part by grant AM-27328 from the US Public Health Service.
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β-Alanine and α-L-alanine inhibit the exploratory activity of spontaneously hypertensive rats[1]

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Summary. 1% β-alanine and α-L-alanine, when given for 7 days as the only drinking fluid, inhibited the exploratory activity of adult male spontaneously-hypertensive rats (SHR) but not that of the normotensive Wistar-Kyoto rats (WKR). β-Alanine decreased the taurine level in the liver of both strains and in the platelets of SHR. α-Alanine decreased the taurine level in the liver of WKR and in the platelets of SHR.

β-Alanine is a structural analog of taurine. It has been shown that β-alanine but not α-L-alanine inhibits the uptake of taurine into the platelets, cortical synaptosomes and retina[2,3]. β-Alanine also inhibits the transport of taurine into the isolated heart[4].

$H_2N-CH_2-CH_2-SO_3H$
Taurine

$H_2N-CH_2-CH_2-COOH$
β-Alanine

$H_2N-CH(CH_3)-COOH$
α-L-Alanine

We have previously shown that taurine enriched and taurine deficient diets stimulate the exploratory activity of SHR but not that of WKR. Both these treatments were assumed to increase the availability of taurine[5]. The present work was designed to study the actions of β-alanine and α-L-alanine in the open field situation and to evaluate the taurine levels in various tissues after alanine administration.

Materials and methods. Male rats of spontaneously hypertensive (SHR) (n = 24; weight mean ± SD 347 ± 17 g) and of normotensive strains of Wistar-Kyoto rats (WKR) (n = 24; weight 312 ± 8 g) were used in the study at the age of 3 months. The rats were housed in groups of 8 with food and fluid ad libidum and maintained on a 12-h light (7.00–19.00 h) -dark cycle.

Taurine content following 7 days' treatment with 1% β-alanine and α-L-alanine

Taurine content (μmole/g; mean ± SD)	Spontaneously hypertensive rats Control	β-Alanine	α-L-alanine	Normotensive Wistar-Kyoto rats Control	β-Alanine	α-L-alanine
Brain	3.5 ± 1.0	3.7 ± 0.5	4.1 ± 0.7	3.7 ± 0.8	3.7 ± 0.8	4.0 ± 0.7
Heart (left ventricle)	24.0 ± 1.9	22.6 ± 1.5	24.9 ± 2.6	25.0 ± 1.7	24.3 ± 3.9	26.8 ± 2.4
Liver	7.2 ± 1.7	3.9 ± 1.5***	8.2 ± 1.6	10.7 ± 0.8	7.1 ± 2.5**	8.5 ± 2.4*
Platelets (μmole/10^{12} cells)	175.1 ± 24.8	139.8 ± 15.9**	125.6 ± 16.0***	152.6 ± 27.1	137.8 ± 58.8	137.7 ± 28.1

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ in comparison to the control group.

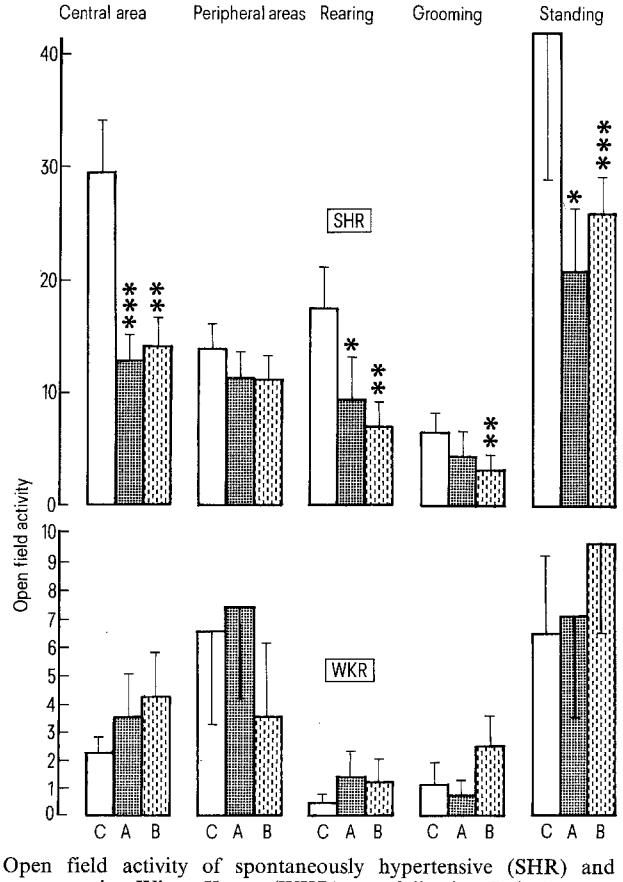
The exploratory activity was measured for 15 min between 19.00 and 22.00 h by using the open field situation[5,6]. 5 different activities were measured; forward locomotion, rearing, grooming, standing and defecation. The locomotion was measured by recording the number of areas crossed. β-Alanine and α-L-alanine were given to the animals in their drinking water as 1% solutions for 7 days. Endogenous brain taurine was estimated as a fluorescamine (Fluram®, F. Hoffman-La Roche) derivative after purification on ion-exchange columns. Brain 5-hydroxytryptamine (5-HT) level was measured spectrophotofluorometrically[7].

Results. All the activity measurements from the open field (figure) indicated that the SHR were more active than the WKR. There was a significant effect for strain in the 2-way analysis of variance for strain and treatment ($F = 58.8$ to 8.10; $p < 0.001$ −0.01 for all parameters). The treatment effect was significant in the SHR for the activity in the central area ($F = 5.4$; $p < 0.01$). Both β-alanine and α-L-alanine decreased the penetrations to the central area. The rearing and standing frequency of the SHR was decreased following both β-alanine and α-L-alanine.

Brain taurine levels did not show any differences due to strain or treatments (table). The taurine level of the liver was significantly higher in the WKR control group than in the SHR control group. Following β-alanine there was a decrease in the liver taurine content. A similar effect was found in the WKR strain after β-alanine and α-L-alanine. A significant decrease in the taurine level of the platelets was seen in the SHR strain after both β-alanine and α-L-alanine. This effect was not evident in the WKR strain. No differences were found in the 5-HT content due to either strain or treatments.

Discussion. In this work the exploratory activity of the SHR was greater than that of the controls. In general SHR are known to be more active[8], to respond more frequently in the Sidman avoidance situation[9], and after isolation to be more aggressive than the normotensive rats[10]. We have earlier shown that the taurine level of platelets in the SHR strain is higher than that of the controls[11], and that a taurine rich diet increases the exploratory activity of the SHR strain[5]. Consequently, the opposite action by the taurine transport inhibitor[12], β-alanine could be expected.

However, α-L-alanine was also able to decrease the taurine level of platelets and liver. Since α-L-alanine does not inhibit taurine uptake[2,3] it is possible that its taurine depleting action is due to an intracellular displacement of taurine from its binding sites. This could lead to a reduction of the functional taurine – even when it is not shown in the total tissue taurine estimations. In WKR the alanine action was opposite to that found in SHR in all respects. This is in agreement with our previous results that taurine treatment affects the behavior of these 2 strains differently[5].



Open field activity of spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKR) rats following 7 day's treatment with β-alanine (B) and α-L-alanine (A); C, control group. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ in comparison to the control group.

1 This study was supported by the Sigrid Jusélius Foundation.

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