Diet-induced antisecretory factor prevents intracranial hypertension in a dosage-dependent manner

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Abstract

Intake of specially processed cereal (SPC) stimulates endogenous antisecretory factor (AF) activity, and SPC intake has proven to be beneficial for a number of clinical conditions. The aim of the present study was to investigate the dosage relationship between SPC intake and plasma AF activity and to further correlate achieved AF levels to a biological effect. SPC was fed to rats in concentrations of 5, 10 or 15 % for 2 weeks. A further group was fed 5 % SPC for 4 weeks. AF activity and the complement factors C3c and factor H were analysed in plasma after the feeding period. Groups of rats fed the various SPC concentrations were subjected to a standardised freezing brain injury, known to induce increases in intracranial pressure (ICP). The AF activity in plasma increased after intake of SPC, in a dosage- and time-dependent manner. The complement factors C3c and factor H increased in a time-dependent manner. Measurements of ICP in animals fed with SPC prior to the brain injury showed that the ICP was significantly lower, compared with that of injured rats fed with a standard feed, and that the change was dose and time dependent. AF activity increases, in a dosage- and time-dependent manner, after intake of SPC. The inverse relationship between ICP after a head injury and the percentage of SPC in the feed indicate that the protective effect is, to a large extent, due to AF.

Key words: Antisecretory factor activation: Complement factors: Intracranial pressure: ELISA: Inflammation

Antisecretory factor (AF), also named S5a, is a 43 kDa ubiquitously expressed protein originally identified through its capacity to inhibit intestinal hypersecretion[1]. Further studies have shown that AF is also a potent anti-inflammatory agent[2–6]. In the plasma of healthy individuals, the major part of AF is present in an inactive form[7]. AF is activated through intestinal exposure to bacterial toxins or through the intake of specific food components, e.g. in the form of specially processed cereals (SPC)[7–9]. Clinical studies have shown that a diet-induced increase in AF activity is beneficial for a number of diseases in which inflammation and/or secretory disturbances are parts of the pathophysiology[6,8,9].

The mechanisms through which SPC activate AF are currently unknown, although exposure of the intestinal mucosa to SPC, in certain aspects, may be assumed to mimic that of intestinal exposure to bacterial toxins, i.e. by inducing an inflammatory response. Such a response could be a first step in the activation process. In a previous study, proteomics was used to study the differences in protein expression in the livers of rats fed an SPC feed and rats fed a standard feed[10]. A selective increase in the expression of glutathione-*S*-transferase μ in the livers of rats fed the SPC feed was observed. Glutathione-*S*-transferase μ does not appear to be involved in AF activation, and the increased expression is probably an unrelated effect of the SPC feed.

In a recent study, in which rats were exposed to blast injury to the head, it was found that pre-treatment with a feed containing SPC prevented the animals from developing high intracranial pressure (ICP), otherwise induced by this type of injury[11]. However, only one concentration of SPC was used and no analyses of AF activity were made. It was unclear whether the effect on ICP was due to AF or if other effects of the SPC feed also contributed.

The aim of the present study was to investigate the time and dose relationships between SPC intake and plasma AF activity and to investigate whether the level of AF activity could be correlated with a biological effect. Rats were fed with different concentrations of SPC for 2 or 4 weeks and the AF activity in plasma was analysed. Furthermore, analyses of the complement factors C3c and factor H were carried out in order to investigate if the SPC feed affected the complement system. A number of animals from each feed group were

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subjected to a closed head injury, known to induce a high ICP. ICP was measured at 2 d after the insult, and the obtained values were correlated with the percentage of SPC in the feed.

Material and methods

Animals

Male Sprague–Dawley rats, having a body weight of 180 (SEM 20) g (Harlan Laboratories), were used. After arrival, the rats were given a week for general adaptation, with free access to pelleted feed and water. The temperature and air ventilation in the animal quarters were monitored and controlled, according to standard procedures. The number of animals in each experimental group is presented in the figures and figure legends.

The test protocol was approved by the Regional Animal Experiments Ethical Committee and performed in accordance with guidelines for animal experiments (EC Directive 86/609/EEC).

Feed

Endogenous formation of the protein AF was stimulated in adult rats by mixing 5, 10 or 15 % SPC (SPC Flakes®; Lantmännen AS-Faktor AB) to standard rat feed (Lab For R36; Lantmännen). The feed, prepared with SPC and R36, was mixed, pelleted and processed in the same way as the R36 feed, therefore, not differing in appearance. Each batch was coded and quality controlled by the Lantmännen standard procedure. The composition of the feeds is given in the work by Johansson *et al.*[10].

Chemicals

Isoflurane was purchased from Baxter Medical AB; all other chemicals were obtained from Sigma-Aldrich. Monoclonal antibodies against the AF protein were produced and characterised in-house[7]. The rabbit anti-C3c polyclonal antibody was purchased from Dako and the rabbit anti-factor H polyclonal antibody from Santa Cruz Biotechnology (SDS Biosciences). The alkaline phosphatase-conjugated goat anti-mouse IgM antibody and goat anti-rabbit IgG antibody were purchased from Jackson ImmunoResearch Europe Limited.

Feed-induced stimulation of antisecretory factor formation

A total of ten groups of rats were fed with the control R36 feed or with different concentrations of SPC mixed with R36: two groups were fed R36 for 2 weeks; two groups, 5 % SPC for 2 weeks; two groups, 10 % SPC for 2 weeks; and two groups, 15 % SPC for 2 weeks. Two groups were fed 5 % SPC for 4 weeks. One group of animals from each feeding schedule was used for ELISA analyses. One group from each feeding schedule was subjected to a head injury and subsequent ICP measurements.

Determination of plasma antisecretory factor activity

The AF activity in purified plasma was determined by means of an ELISA method developed in-house, as previously reported[7]. The AF activity in the purified samples was determined, using the monoclonal antibody 3H8[7] as the detecting antibody. The bound enzyme in the assay was revealed by absorbance at 405 nm. Data are presented as means with their standard errors.

ELISA determination of complement factors C3c and factor H

The levels of C3c and factor H in the affinity-purified rat plasma samples were determined by an ELISA method, using rabbit anti-C3c or anti-factor H polyclonal antibody as the detecting antibody. A second antibody of goat anti-rabbit IgG-alkaline phosphatase conjugate was then applied, followed by absorbance readings at 405 nm, to reveal the bound enzyme. Rabbit pre-immune serum was used as the background and the values were deducted to give net absorbances. Data are presented as means with their standard errors.

Surgical procedure and induction of a traumatic brain injury

A modification of the method described by Schneider *et al.*[12] was used. The operation procedure started by inhalation of isoflurane, after which the anaesthetised rat was positioned on the abdomen, on a heating pad, the head shaved and a midline incision made through the skin and fascia on the skull vault. The calvarium was freed of adherent tissue and a Cu rod (144 g) with a tip diameter of 4 mm, kept immersed in liquid N_2, was then applied to the right parietal bone for 45 s. This freezing procedure induced a superficial injury to the underlying brain tissue. The midline of the cranium was avoided in order to not induce any damage to the superior sagittal sinus. The wound was then sutured, the rat received an analgesic and recovered from the anaesthesia. All animals survived the anaesthesia and the operation procedure, and none showed any signs of subsequent impairment. Body temperatures were monitored with a rectal probe (Panlab temp control unit HB 101/2; Harvard Apparatus) and blood pressures were measured by intermittent cuffing of tail vessels (LE 5002; Panlab).

Measurement of intracranial pressure

ICP was measured, under isoflurane anaesthesia, at 2 d after the traumatic brain injury, as previously described[13]. In short, two light emitting diode (LED) fibre optic pressure transducers (Samba Sensors AB) connected to a control unit (Samba 3200) and a computer were inserted into the right and left hemispheres of the brain through two small holes (diameter 1 mm). Two pressure transducers were used to check the accuracy of the measurements. A similar ICP recorded from both sides indicate that the system is functioning properly.

The diameter of the optic fibre was 0·25 mm and that of the sensor was 0·42 mm. The pressure sensors were factory calibrated and the accuracy of the readings checked prior to

and after the experiments. The calibration procedure was performed by immersion of the sensors in water-filled glass cylinders of known dimensions. Additionally, during the experimental periods, the probes were calibrated intermittently against the ambient atmospheric pressure, using the software supplied by the manufacturer. Data are presented as means with their standard errors. The animals were killed after the registration period.

Statistics

Values are expressed as means with their standard errors. In order to stabilise variances, values were logarithm-transformed using the natural logarithm. For investigation of dosage–response relationships after 2 weeks of feeding, a variance analysis followed by Turkey's test for multiple comparisons were used. For each analysis performed (ICP, AF, C3c and factor H), paired comparisons were made between all groups fed the various percentages of SPC. A value of P less than 0·05 was considered to be statistically significant. For comparison of the two groups fed 5% SPC for 2 and 4 weeks, respectively, Student's two-tailed t test for independent samples was used. A value of P less than 0·05 was considered to be statistically significant. SPSS software (version 18; SPSS) was used for statistical evaluation.

Results

Effects of 5, 10 and 15% specially processed cereals on rat plasma antisecretory factor activity

In animals fed the various SPC concentrations for 2 weeks, the plasma levels of active AF increased with increasing percentage of SPC (Fig. 1). A significant increase was induced by the 10 and 15% SPC feeds ($P<0·01$ and $P<0·001$,
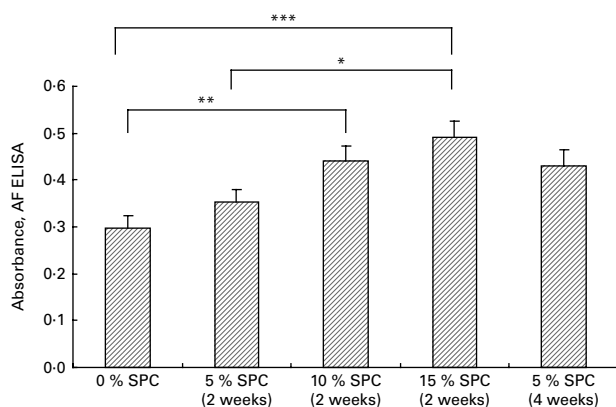

Fig. 1. The levels of antisecretory factor (AF) activity in affinity-purified rat plasma determined by ELISA using AF monoclonal antibody 3H8. The graph demonstrates the absorbance levels in samples from rats fed control feed with 0% specially processed cereal (SPC) (n 10), rats fed with 5% SPC for 2 weeks (n 9), with 10% SPC for 2 weeks (n 9), with 15% SPC for 2 weeks (n 9) and with 5% SPC for 4 weeks (n 13). Values are means, with their standard errors represented by vertical bars. There was a significant increase in plasma AF levels after intake of 10 and 15% SPC for 2 weeks, as shown in the figure. The difference in AF levels between 2 and 4 weeks' intake of 5% SPC was statistically significant ($P<0·05$). Mean values were significantly different: * $P<0·05$; ** $P<0·01$; *** $P<0·001$.
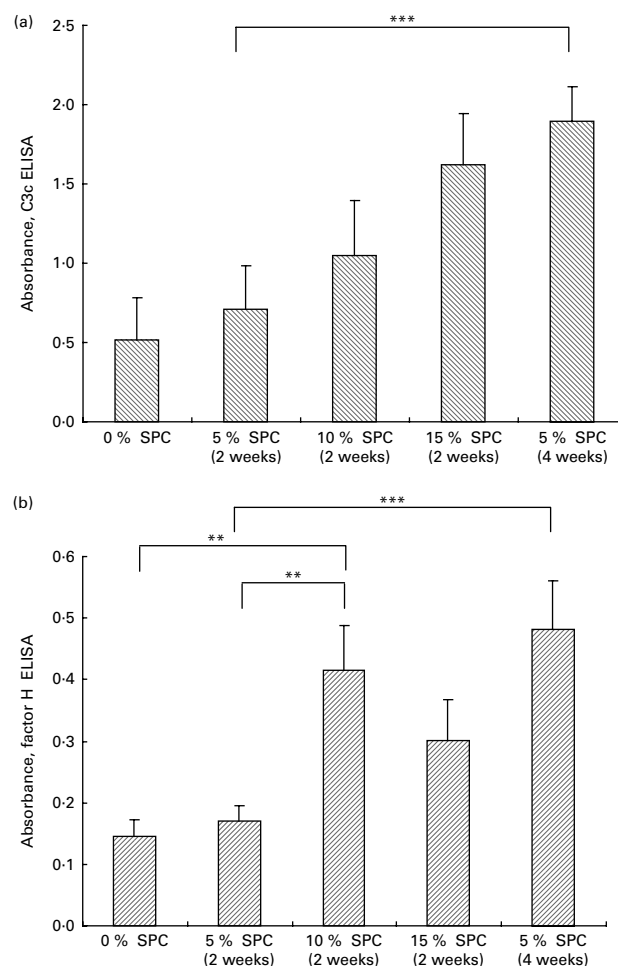

Fig. 2. The levels of (a) complement factor C3c and of (b) factor H in affinity-purified plasma, from the same animals as in Fig. 1, analysed by ELISA. Values are means, with their standard errors of net-absorbance levels represented by vertical bars. For both complement factors, the plasma concentration was significantly higher after 4 weeks of 5% SPC than after 2 weeks of 5% SPC. Factor H levels were significantly increased after 10% SPC for 2 weeks compared with the initial level. Mean values were significantly different: ** $P<0·01$, *** $P<0·001$.

respectively), but not by the 5% SPC feed. AF activity after 4 weeks of feeding with 5% SPC was slightly higher than after a 2-week feeding period ($P<0·05$).

Effects of 5, 10 and 15% specially processed cereals on complement factors C3c and factor H

Complement factors C3c and factor H were used as biomarkers for immune/inflammatory activation after dietary intake of SPC[14] (Fig. 2). C3c levels after 2 weeks of feeding with the various SPC concentrations did not significantly differ from that in animals fed the control feed for any of the SPC concentrations. However, extending the feed period with 5% SPC to 4 weeks resulted in a significant increase in the C3c level compared with that obtained after 2 weeks ($P<0·001$) (Fig. 2(a)).

The levels of factor H were significantly increased after intake of 10% SPC for 2 weeks ($P<0·01$) (Fig. 2(b)). The

factor H level after 4 weeks of feeding with 5% SPC was significantly higher than after 2 weeks of feeding with 5% SPC ($P<0.001$).

Effects of 5, 10 and 15 % specially processed cereals on intracranial pressure in normal rats and after brain injury

ICP values are shown in Fig. 3. ICP in uninjured rats fed with the control feed R36 was 6·2 (SEM 0·2) mmHg in the left hemisphere and 6·3 (SEM 0·2) mmHg in the right hemisphere. As the ICP difference between the right and left hemispheres was not significant in any of the groups of animals, the mean of the two pressures were used for the statistical calculations.

Brain injury resulted in an increased ICP at 2 d after the insult in all groups of animals ($P<0.001$ v. uninjured controls). The highest levels were obtained in the group of rats fed the control R36 feed (22·9 (SEM 1·6) and 25·4 (1·4) mmHg, in the right and left hemispheres, respectively). In all groups of animals treated with the various SPC feeds for 2 weeks before the injury, ICP was significantly lower than that in the group fed the control feed.

In the group fed with 5% SPC for 2 weeks, ICP was significantly lower, compared with the group fed the control feed ($P<0.05$), and a further decrease in ICP was seen in animals fed the 10% SPC feed ($P<0.001$, 5% SPC v. 10% SPC). Increasing the percentage of SPC in the feed to 15% had no further effect on the measured ICP.

If the feeding period with 5% SPC before the injury was extended to 4 weeks, ICP at 2 d after injury was significantly lower than in animals fed 5% SPC for 2 weeks ($P<0.001$).

Discussion

Induction of AF activity occurs as a response to intestinal exposure of bacterial toxins, e.g. cholera toxin[15]. The increased AF activity is part of the natural response to diarrhoeal diseases and contributes to the healing process. This naturally occurring activation of AF can be mimicked by the intake of specific feed components consisting of a mixture of sugars and amino acids[16]. Such a mixture is obtained in SPC, and intake of SPC is followed by increased AF activity in plasma[7]. In previous human studies, a standardised dose of SPC based on body weight was used[8,9,17]. We now show that induced AF levels are related to the percentage of SPC in the feed as well as to the period of intake.

The biological significance of an increased AF activity for brain function was demonstrated by evaluating the response to a closed head injury. Thus, rats fed SPC for at least 2 weeks prior to the injury were protected from the ensuing ICP increase in a dose- and time-dependent manner. The present finding of a graded response correlated with the SPC percentage in the feed indicate that AF is responsible for a major part of the prevention of ICP increase. However, other effects of SPC unrelated to AF activity cannot be excluded[10].

The exact activation steps for AF are unknown, although both modes of induction, i.e. exposure to bacterial toxins or intake of feed components, probably occur through similar pathways. In the present study, plasma levels of the complement factors were observed to increase in a time-dependent manner after intake of SPC. It appears that SPC, possibly as an
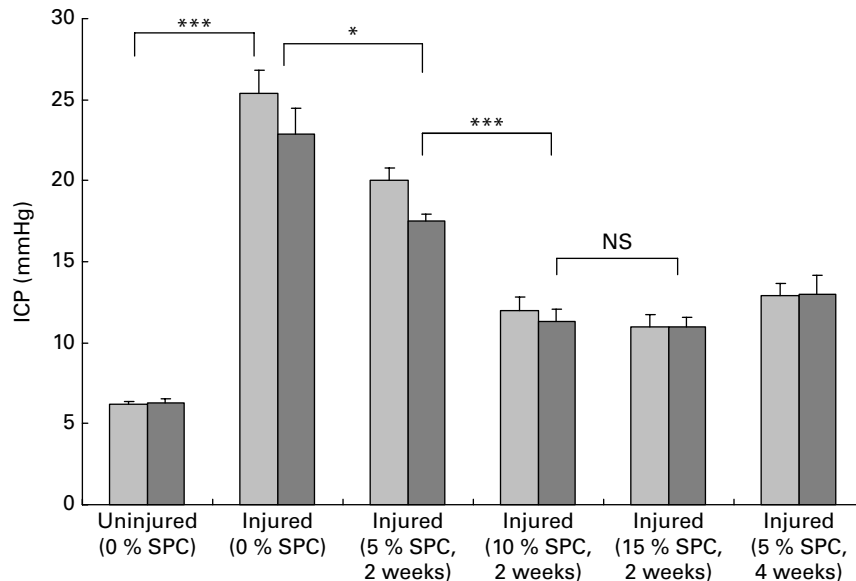

Fig. 3. Intracranial pressure (ICP) in non-injured rats and in rats subjected to a freeze-induced, right-sided closed brain trauma. The values were registered 2 d after injury, and ICP determined simultaneously in the left (▢) and right (▮) brain hemispheres. The mean ICP during the time period 27–180 min after implantation of the pressure recording probes in the occipital lobes are shown. Values are means, with their standard errors of net absorbance levels represented by vertical bars. The ICP of uninjured control rats fed control feed with 0% specially processed cereal (SPC) (n 24) was low and similar in both hemispheres. The ICP in all injured rats were significantly higher than in the uninjured control rats ($P<0.001$). The animals fed the control feed (n 21) showed the highest values. Successively decreasing values were registered in injured animals fed 5% SPC for 2 weeks (n 14) and 10% SPC for 2 weeks (n 8), but there was no further decrease in ICP in animals fed 15% SPC for 2 weeks (n 10). The ICP of rats fed 5% SPC for 4 weeks (n 19) was significantly lower than the ICP of the rats fed 5% SPC for 2 weeks ($P<0.001$). Mean values were significantly different: * $P<0.01$, *** $P<0.001$.

early step in the activation process, induces an inflammatory reaction, which is reflected in the plasma levels. Cholera toxin, a potent activator of AF[15,16], induces only a mild inflammatory reaction[18], although it may be sufficient for the activation process.

The mechanism of action for AF is unknown. Previous studies have shown that endogenously produced protein AF and exogenously administrated AF peptides have similar effects in several experimental systems[19]. Taken together, the results of previous experimental and clinical studies indicate that the effects of AF are, to a large extent, exerted through inhibition or modulation of inflammation[2,3,6,20,21]. In a recent study, constitutive mRNA levels of AF were found to vary between mouse strains, and a higher amount of AF due to genetic background may contribute to resistance to brain injury by counteracting inflammation[22]. A possible sequence of events could be that intake of SPC induces a mild inflammatory reaction, which is accompanied by formation of active AF. When formed, active AF protein acts as an anti-inflammatory agent.

The injury model used in the present study results in a combined cytotoxic and vasogenic oedema[12], of which inflammation is probably a prominent feature[23,24]. It is tempting to suggest that AF exerts its anti-inflammatory action by inhibiting release of pro-inflammatory cytokines from cell populations present in most tissues, e.g. mast cells[25] or macrophages/microglia[26]. A further effect of traumatic brain injury, contributing to inflammation and brain oedema, is an increase in NO due to the expression of inducible NO synthase[27]. Inhibitor of NOS, e.g. VAS203[28], has been shown to decrease brain oedema and intracranial hypertension after experimental traumatic brain injury. The peak in inducible NO synthase induction occurs between 6 and 72 h after a brain injury[26], which coincides with the time point at which the ICP measurements were made in the present study. Further studies are needed to investigate if inducible NO synthase/NO effects can be modified by AF.

The present results demonstrate that AF activity in plasma increases in a dose- and time-dependent manner after intake of SPC. The SPC-induced AF activation process involves an inflammatory reaction. The data also indicate a dose–response relationship between the level of active AF and its biological effects in vivo. If the present results are applicable also for human subjects, they allow for a more individualised AF treatment in future studies.

Acknowledgements

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