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European Journal of Pharmacology 374 (1999) 207–211

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Short communication

4-(2-Aminoethyl)benzenesulfonyl fluoride attenuates tumor-necrosis-factor-α-induced blood–brain barrier opening

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Received 10 December 1998; received in revised form 25 March 1999; accepted 30 March 1999

Abstract

The effect of serine protease inhibitor 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF) was investigated on the prevention of tumor-necrosis-factor-α (TNF-α)-induced blood–brain barrier opening. TNF-α (10,000 IU) was injected intracarotidly to newborn pigs pretreated with 0, 2.4, 4.8, 9.6 and 19.2 mg/kg AEBSF ($n = 6$ in each group). AEBSF dose-dependently inhibited the TNF-α-induced increase in the blood–brain barrier permeability for sodium fluorescein (MW = 376) in all of the five brain regions examined, while only 19.2 mg/kg AEBSF could significantly ($P < 0.05$) decrease the change in Evan's blue-albumin (MW = 67,000) transport in two regions. In conclusion, AEBSF attenuates vasogenic brain edema formation. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: AEBSF [4-(2-aminoethyl)benzenesulfonyl] fluoride; Blood–brain barrier; Brain edema; Serine protease inhibitor; TNF-α (tumor necrosis factor-α)

1. Introduction

Tumor necrosis factor-α (TNF-α) plays a crucial role in the development of acquired immunodeficiency syndrome, malaria, meningococcal disease, parasitic infections and sepsis (Beutler and Grau, 1993; Feuerstein et al., 1994) and it proved to be an important mediator of brain injury during cerebral ischemia–reperfusion (Del Zoppo, 1994) and neurodegenerative diseases (Sharief and Thompson, 1992). The blood–brain barrier, formed by cerebral endothelial cells in cooperation with astrocytes, neurons, pericytes and microglia, plays an active role in TNF-α-induced cerebral damage. Stimulated cells located at either side of this barrier can produce TNF-α which induces capillary endothelial cell proinflammatory responses (e.g., leukocyte–endothelial adhesion, neutrophil transmigration), and increased procoagulant activity by the activation of some serine proteases (Beutler and Grau, 1993; Del Zoppo, 1994; Feuerstein et al., 1994). On the other hand, TNF-α has a specific bidirectional transport system through which it passes the blood–brain barrier (Gutierrez et al., 1993).

Previous studies indicated that both intravascular and intracisternal TNF-α administration resulted in vasogenic brain edema formation in newborn pigs (Megyeri et al., 1992; Ábrahám et al., 1996). It is assumed that activated leukocytes contribute to the acute blood–brain barrier opening, because no similar changes in permeability were found in an in vitro reconstituted model of the blood–brain barrier (cerebral endothelial cells cocultured with astrocytes) (Deli et al., 1995). The vasoconstrictor effect of intracisternal TNF-α (Megyeri et al., 1992; Tureen, 1995) suggests the involvement of cerebrovascular smooth muscle cells, too. It is known that oxygen free radicals produced by endothelial cells, brain cells or white blood cells play a role in the mediation of the TNF-α-induced brain injuries (Del Zoppo, 1994; Feuerstein et al., 1994; Tureen,

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PII: S0014-2999(99)00224-1

1995). Our recent data suggested that TNF-α maintains high superoxide production of human monocytes in vitro which was blocked by the addition of 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), a nontoxic, water-soluble, irreversible inhibitor of serine proteases (Megyeri et al., 1995). Similarly, the in vitro activation of respiratory burst in stimulated neutrophil granulocytes could also be prevented by AEBSF pretreatment (Remold-O'Donnell and Parent, 1995).

The aim of the present study was to reveal the possible in vivo effect of AEBSF on the prevention of TNF-α-induced vasogenic brain edema formation.

2. Materials and methods

2.1. Animal study

Newborn pigs of either sex ($n = 36$, 4–8 h, 1170–1580 g) were included. After pentobarbital (30 mg/kg) anesthesia, one of the umbilical arteries was catheterized, and cardiovascular, blood gas and acid–base parameters were monitored (Ábrahám et al., 1996). The left internal carotid artery of the animals was catheterized through the external branch, and 10,000 IU rhTNF-α diluted in 0.5 ml isotonic saline was given in slow intraarterial injection to 30 animals, while six newborn pigs receiving isotonic saline served as control. In the drug study, 1 h before the TNF-α administration the animals ($n = 6$ in each group) were pretreated intravenously with 0, 2.4, 4.8, 9.6 and 19.2 mg/kg AEBSF (MW = 239.5) diluted in 1 ml isotonic saline. The experimental procedures followed the National Institute of Health (Bethesda, MD, USA) Guidelines for the care and use of laboratory animals and were approved by the local Ethical Committee on Animal Investigation.

2.2. Blood–brain barrier permeability measurements

The development of vasogenic brain edema was measured 4 h after the TNF-α challenge using 2 intravascular blood–brain barrier permeability tracers: sodium fluorescein (MW = 376) and Evan's blue labelled albumin (MW = 67,000), as it was previously described in details (Ábrahám et al., 1996). Sera as well as perfused tissue samples from both ipsilateral (left hemisphere) and contralateral (right hemisphere) parietal cortex, hippocampus, striatum, periventricular white matter and cerebellum were homogenized in 3.0 ml of cold 7.5% trichloroacetic acid and centrifuged at $10{,}000 \times g$ for 10 min. Evan's blue (absorbance: 620 nm) and sodium fluorescein (excitation: 440 nm, emission: 525 nm) concentrations were determined by a Hitachi F 2000 fluorimeter (Tokyo, Japan). Extravasation was expressed as brain tissue concentration divided by serum concentration for both tracers.

2.3. Drugs and chemicals

All drugs and chemicals used in this study were purchased from Sigma (St. Louis, MO, USA).

2.4. Statistical analysis

All data presented are means ± S.E.M, $n = 6$ in each group. The intergroup values were compared using Kruskal–Wallis one-way analysis of variance on ranks followed by multiple comparisons with Student–Newman–Keuls test. The differences between ipsi- and contralateral hemispheres were evaluated by Mann–Whitney rank sum test. Changes were considered statistically significant at $P < 0.05$.

3. Results

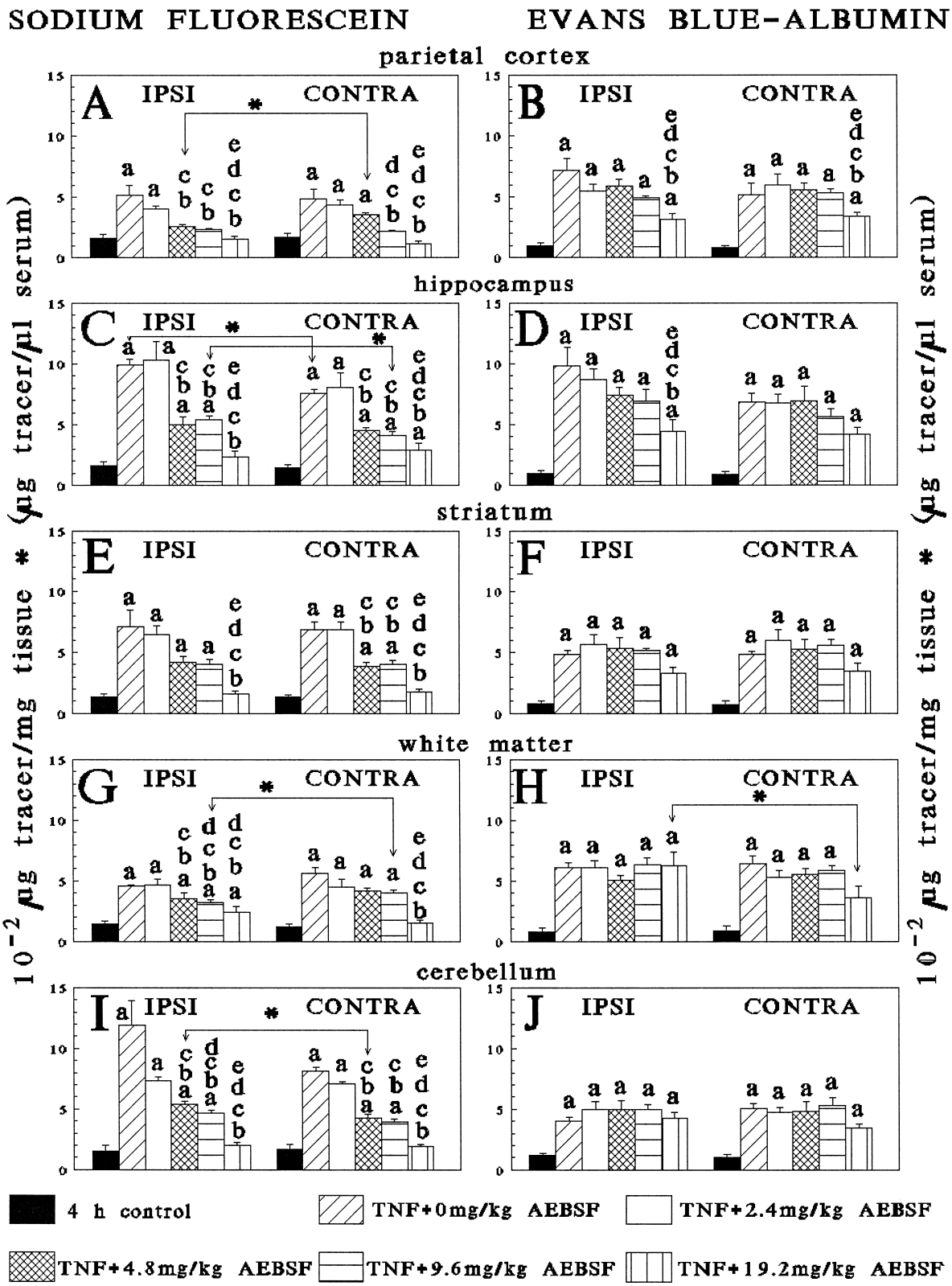
During the experimental period vital cardiovascular parameters did not change significantly after administration of TNF-α and AEBSF compared to those measured in control animals (data not shown).

On the other hand, 10,000 IU rhTNF-α resulted in blood–brain barrier opening both for sodium fluorescein and albumin in porcine brain (Fig. 1). AEBSF pretreatment, in the doses of 4.8–19.2 mg/kg, significantly ($P < 0.05$) inhibited the TNF-α induced increase in sodium fluorescein permeability in all regions (Fig. 1A,C,E,G,I). The effect was dose-dependent and the highest dose could prevent the opening of the barrier for this intravascular tracer. However, the effect of AEBSF on the increased albumin permeability was less expressed: only the dose of

Fig. 1. Effect of AEBSF pretreatment on the blood–brain barrier permeability 4 h after intracarotid challenge with 10,000 IU TNF-α. Filled bars: control animals, while the other columns represent the five TNF-α-treated groups according to the doses of AEBSF applied: 0 mg/kg (hatched bars), 2.4 mg/kg (open bars), 4.8 mg/kg (cross-hatched bars), 9.6 mg/kg (horizontal line bars), and 19.2 mg/kg (vertical line bars), respectively. Cerebrovascular permeability was determined in parietal cortex (A,B), hippocampus (C,D), striatum (E,F), periventricular white matter (G,H), and cerebellum (I,J) of newborn pigs. The permeability markers were: sodium fluorescein (A,C,E,G,I) and Evan's blue-albumin (B,D,F,H,I). Extravasations were expressed as 10^{-2} μg dye/mg brain tissue × (μg dye/μl serum)$^{-1}$ for both dyes. Letters a–e indicate significant differences ($P < 0.05$) compared to values measured (a) in control group, as well as in TNF-α-treated groups receiving the following doses of AEBSF: (b) 0 mg/kg; (c) 2.4 mg/kg; (d) 4.8 mg/kg; and (e) 9.6 mg/kg, respectively. Symbol * shows significant difference between permeability of ipsi- and contralateral sides of the same brain region.

19.2 mg/kg AEBSF could significantly ($P < 0.05$) decrease the extravasation in parietal cortex of both sides (Fig. 1B), as well as in ipsilateral hippocampus (Fig. 1D).

Comparing cerebrovascular permeability measured in the same region of two hemispheres, statistically significant ($P < 0.05$) differences were only seen in five cases for



sodium fluorescein (Fig. 1A,C,G,I) and one case for Evan's blue-albumin (Fig. 1H).

4. Discussion

In accordance with previous observations (Ábrahám et al., 1996), our present data confirm that intracarotid TNF-α administration results in blood–brain barrier opening for sodium fluorescein and Evan's blue-albumin in the brain of newborn pigs. Though AEBSF pretreatment dose-dependently inhibited the TNF-α-induced barrier opening for sodium fluorescein, its effect on the increase in albumin permeability was moderate. However, it is suggested that permeability tracers used can pass through the blood–brain barrier by different permeation pathways: sodium fluorescein through the opened interendothelial tight junctions (Thompson et al., 1994), while albumin by transcytosis (Banks and Broadwell, 1994). It is hypothesized that AEBSF in the doses used may primarily act on the permeability by the prevention of disruption of tight junctions and not by attenuating transendothelial transport. In accordance with this presumption, Winton et al. (1998) found that AEBSF inhibited the serine protease-induced breakdown of tight junctions of epithelial monolayers and significantly decreased the paracellular permeability. Similarly, Nagy et al. (1995) published that thrombin, plasmin, or urokinase could cause the contraction of brain endothelial cells which might result in increased paracellular blood–brain barrier permeability. Moreover, TNF-α induces serine protease gelatinase B, a matrix metalloproteinase, which can cause delayed opening of the blood–brain barrier (Rosenberg et al., 1995).

It is known that free radicals are also involved in the mediation of the TNF-α induced brain injuries (Del Zoppo, 1994; Feuerstein et al., 1994; Tureen, 1995). We assume that AEBSF may decrease the development of brain edema, at least partly, by blocking the serine protease responsible for the proteolysis of the inhibitory protein Iκβ-α, thereby preventing the activation of nuclear factor-κβ (NF-κβ) and inhibiting the free radical production of activated cells (Megyeri et al., 1995; Remold-O'Donnell and Parent, 1995; Hecker et al., 1996).

Serine protease inhibitors may have a therapeutical role in the treatment of infections, ischemia–reperfusion injuries, and trauma in the near future. Recent reports have revealed some beneficial effects of AEBSF on brain cells: it could inhibit amyloid κ-protein production (Citron et al., 1996), and could protect against glutamate-induced programmed cell death (Tan et al., 1998) in neuronal cell lines. This report is the first to describe AEBSF, a potent and stable serine protease inhibitor to reduce the TNF-α-induced vasogenic brain edema formation. However, further studies are warranted to elucidate the importance of serine proteases and their inhibitors in the regulation of the blood–brain barrier permeability.

Acknowledgements

The research was partly supported by grants from OTKA (T-2680, F-16682, F-25984), ETT (T07 154/96), and INSERM (réseau Est-Ouest 94EO06). The authors are grateful to Mrs. Ildikó Wellinger for her skillful technical assistance.

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