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Effects of *N*,*N*-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine on the blood–brain barrier permeability in the rat

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Abstract

Histamine plays a role in the regulation of the blood–brain barrier function. In this study, effects of *N*,*N*-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine (DPPE), an intracellular histamine binding site antagonist on the cerebrovascular permeability were investigated in control and post-ischemic male Wistar rats. Intravenous administration of DPPE, in a dose of 1 and 5 mg/kg, was not followed by any major clinical change, but 20 mg/kg proved to be toxic. A significantly ($P < 0.05$) increased permeability for sodium fluorescein (MW = 376) was seen in hippocampus, striatum, and cerebellum, but not in parietal cortex, of rats 2 h after the injection of 5 mg/kg DPPE, whereas no increase was measured later. There was a more intense (5- to 12-fold) and prolonged elevation in Evan's blue-labeled albumin (MW = 67,000) extravasation 2, 4, and 8 h after 5 mg/kg DPPE administration in each brain region. In parietal cortex, a dose-dependent increase in albumin extravasation developed 4 h after intravenous injection of 1, 5, and 20 mg/kg DPPE, but doses applied resulted in no significant change in sodium fluorescein permeability. Cerebral ischemia–reperfusion evoked by four-vessel occlusion caused a significant ($P < 0.05$) increase in the permeability for albumin in each region, but few changes in that of sodium fluorescein. DPPE treatment failed to prevent the ischemia–reperfusion-induced changes in the blood–brain barrier permeability. In conclusion, DPPE induced an increased permeability in the rat, which supports a role for histamine, as an intracellular messenger, in the regulation of the blood–brain barrier characteristics.

Keywords: Blood–brain barrier; Brain edema; Cerebral ischemia–reperfusion; Histamine; Intracellular histamine binding site; DPPE (*N*,*N*-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine)

1. Introduction

It has long been known that histamine plays a role in the brain both under physiological and pathological conditions. In the central nervous system, histamine can be released from three cellular compartments, such as histaminergic neurons, perivascular mast cells, and cerebral microvessels (Edvinsson et al., 1993). Histamine can act as a neurotransmitter (Schwartz et al., 1991), and as a regulator of cerebral blood flow (Edvinsson et al., 1993), or blood–brain barrier permeability (Joó, 1996). Different stimuli, such as cryogenic injury (Orr, 1988), brain trauma (Mohanty et al., 1989), focal cerebral ischemia (Adachi et al., 1992), perinatal asphyxia (Kovács et al., 1995), could increase the intracerebral histamine content.

Cerebral endothelial cells possess both histamine type 1 (H_1) and type 2 (H_2) receptors (Karlstedt et al., 1999), but there are no data published about the presence of histamine type 3 (H_3) receptors or intracellular histamine binding sites (Hic) on the cells forming the blood–brain barrier. Histamine is a well-known mediator of brain edema formation (Joó, 1993). Previous studies revealed a predominance of histamine H_2 receptor-dependent, adenylate cyclase-mediated mechanisms in the histamine-induced changes in the cerebrovascular permeability, but histamine H_1 receptor dependent, phosphoinositol-mediated mechanisms could also contribute to this process (Karnushina et al., 1980; Gross et al., 1981; Dux and Joó, 1982; Dux et al., 1987; Butt and Jones, 1992; Edvinsson et al., 1993; Joó, 1993;

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Schilling and Wahl, 1994; Tósaki et al., 1994; Joó, 1996). Recently, Mayhan (1996) published evidences that nitric oxide could participate in the histamine-induced increase in the blood–brain barrier permeability, most probably through the activation of guanylate cyclase.

Isolated cerebral microvessels were shown to have high histamine content, and were suggested to have a low activity of histamine-synthesizing (L-histidine decarboxylase) and histamine-metabolizing (histamine-*N*-methyltransferase) enzymes (Karnushina et al., 1979; Karnushina et al., 1980; Robinson-White and Beaven, 1982). Histamine was assumed not to be synthesized in, but rather taken up by brain capillaries (Joó et al., 1981). Similarly, Karlstedt et al. (1999) failed to prove the histamine-producing capacity of cerebral endothelial cells. Furthermore, a specific high affinity uptake and release system for histamine was described in primary cultures of brain endothelial cells (Huszti et al., 1995, 1997), whereas a non-saturable histamine transport was demonstrated in an immortalized cerebral endothelial cell line (RBE4) (Karlstedt et al., 1999). In RBE4 cells, internalized histamine was distributed not only in cytoplasm, but in the nucleus also, which might support a role to be elucidated for the histamine Hic binding site in the regulation of the blood–brain barrier function (Karlstedt et al., 1999).

After identifying the existence of the novel low affinity (micromolar range) intracellular histamine Hic binding site, Brandes et al. (1990) proposed that histamine might act as an intracellular messenger. Their hypothesis is based on the biological effects of *N,N*-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine HCl (DPPE), a potent inhibitor of this site, which is also a ligand for the microsomal *P*450 antiestrogen binding site. As it was reviewed by Brandes et al. (1990), DPPE inhibits phorbol ester-induced platelet aggregation, has antiproliferative or cytotoxic effects to malignant cells, confers cytoprotection in gastric mucosa, and interacts with low affinity histamine sites in brain membrane fractions. However, the possible contribution of histamine, as an intracellular messenger, to the central nervous system activity remains to be revealed. According to data available, histamine Hic binding site antagonist DPPE has controversial cerebral effects. DPPE potentiated the chemically induced convulsions in mice (Sturman et al., 1994), suppressed *N*-methyl-D-aspartate currents in hippocampus slices (Sharonova et al., 1996), and reduced the cerebral infarction due to focal ischemia in mice (Cramer and Toorop, 1998). Our working hypothesis was that histamine Hic binding site antagonist DPPE, similarly to the histamine H_2 receptor antagonists and some histamine H_1 receptor blockers (Dux et al., 1987; Edvinsson et al., 1993; Joó, 1993; Tósaki et al., 1994) could prevent the cerebral ischemia–reperfusion-induced vasogenic edema formation.

This study was undertaken to investigate the effects of DPPE on the regional permeability of the blood–brain barrier in healthy control rats and post-ischemic animals. After the determination of some basic clinical data, we measured the time- and dose-dependent effects of intravenous DPPE treatment on the extravasation of sodium fluorescein and albumin. The influence of DPPE on the cerebral ischemia–reperfusion-induced cerebrovascular permeability changes was also investigated. A preliminary

Table 1
Effects of cerebral ischaemia–reperfusion and intravenous DPPE administration on the mean arterial blood pressure and arterial blood chemistry parameters of rats
Abbreviations are as follows: MABP, mean arterial blood pressure; pCO_2, partial carbon dioxide tension, pO_2, partial oxygen tension. Values are means ± S.D., $n = 6$ in each group, n.m. = not measured. No significant ($P < 0.05$) differences were found between the values measured in the four experimental groups using one-way analysis of variance.

Experimental group	MABP (mm Hg)	pH	pCO_2 (mm Hg)	pO_2 (mm Hg)	Hematocrit
Control					
0 min	112 ± 14	7.40 ± 0.06	38 ± 8	107 ± 20	n.m
30 min	114 ± 10	7.40 ± 0.05	39 ± 8	105 ± 18	n.m.
60 min	109 ± 13	7.39 ± 0.04	40 ± 6	105 ± 13	0.39 ± 0.01
DPPE (5 mg / kg)					
0 min	110 ± 11	7.39 ± 0.05	38 ± 6	107 ± 19	n.m.
30 min	105 ± 12	7.39 ± 0.05	40 ± 5	106 ± 17	n.m.
60 min	106 ± 15	7.38 ± 0.06	40 ± 5	109 ± 15	0.38 ± 0.04
Ischemia–reperfusion					
0 min reperfusion	109 ± 9	7.37 ± 0.05	44 ± 7	103 ± 14	n.m.
30 min reperfusion	115 ± 13	7.37 ± 0.05	42 ± 7	102 ± 18	n.m.
60 min reperfusion	112 ± 7	7.34 ± 0.05	41 ± 6	101 ± 14	0.37 ± 0.04
DPPE (5 mg / kg) + ischemia–reperfusion					
0 min reperfusion	111 ± 13	7.36 ± 0.07	43 ± 7	99 ± 15	n.m.
30 min reperfusion	107 ± 11	7.37 ± 0.05	41 ± 7	102 ± 20	n.m.
60 min reperfusion	105 ± 14	7.35 ± 0.06	38 ± 7	104 ± 16	0.38 ± 0.04

study (Németh et al., 1998) has already been published about the effect of DPPE on post-ischemic cerebral cortex.

2. Materials and methods

2.1. Animal study

Male Wistar rats (300–350 g) were used in this study. Experimental procedures approved by the local Ethical Committee on Animal Investigation followed the National Institute of Health (Bethesda, MD, USA) Guidelines for the care and use of laboratory animals.

Effects of the intracellular histamine receptor antagonist, DPPE, on the blood–brain barrier permeability was investigated both in control animals and in rats underwent cerebral ischemia–reperfusion. In control rats, permeability was determined 2, 4 and 8 h after receiving slow intravenous injection of 5 mg/kg DPPE ($n = 6$ in each group). We also tested the effect of 0, 1, 5, and 20 mg/kg DPPE dissolved in 0.5 ml isotonic saline on the permeability 4 h after the administration. In the second part of the study, ischemic rats were given intravenous injection of either 0.5 ml vehicle (isotonic saline) or 5 mg/kg DPPE at the beginning of reperfusion, and blood–brain barrier permeability was detected 2, 4 and 8 h later ($n = 6$ in each group). Non-ischemic animals receiving intravenous injection of 0.5 ml isotonic saline 2, 4, and 8 h prior to the permeability measurements served as controls.

Cerebral ischemia and reperfusion was induced according to a modification of the four-vessel occlusion model of Pulsinelli and Brierley (1979), as it was previously described in details (Németh et al., 1998). Animals were anesthesized by intraperitoneal injection of pentobarbital (30 mg/kg). The first cervical vertebra (C1) was approached via a posterior midline muscle-splitting incision and alar foramina was identified. Neurovascular branch containing vertebral artery was coagulated by a bipolar diathermical forceps on both sides. Then, common carotid arteries were isolated on both sides from an anterior

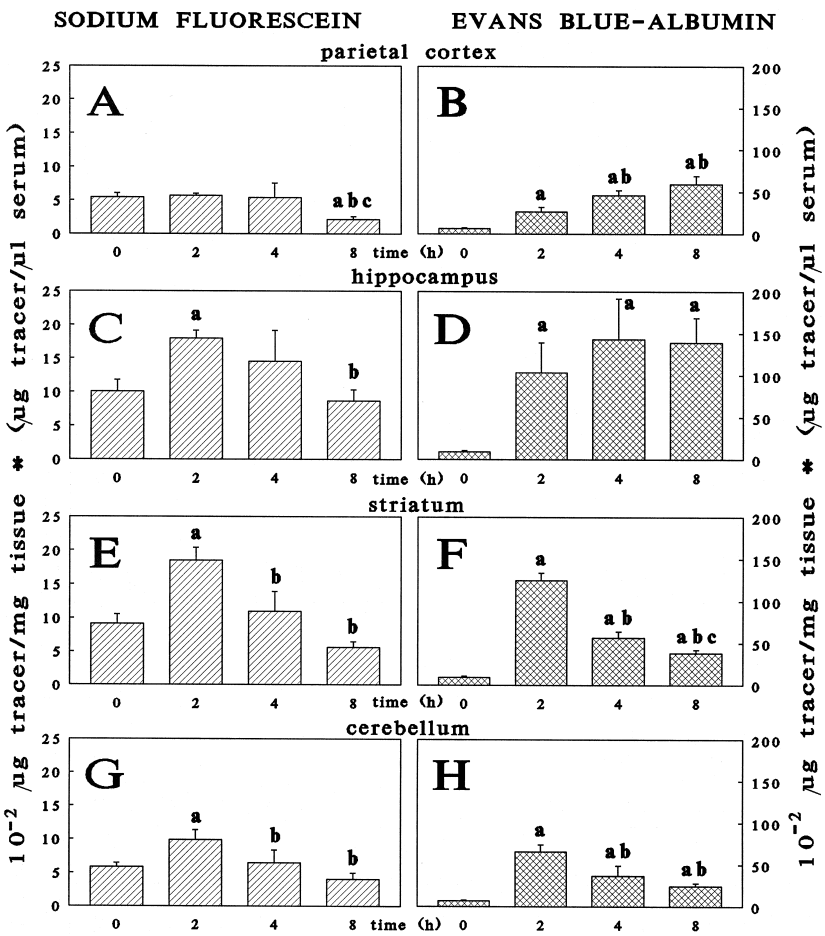

Fig. 1. DPPE on the blood–brain barrier permeability in the rat. Permeability for sodium fluorescein (A,C,E,G) and Evan's blue-albumin (B,D,F,H) was determined in parietal cortex (A,B), hippocampus (C,D), striatum (E,F), and cerebellum (G,H) before and 2, 4, and 8 h after the intravenous administration of 5 mg/kg DPPE. Extravasations were expressed as 10^{-2} μg tracer/mg brain tissue × (μg tracer/μl serum)$^{-1}$ for both dyes, each value presented is a mean ± S.E.M., $n = 6$. Letters indicate significant differences ($P < 0.05$) compared to values measured at the following time-points in the same brain region: (a) 0 h; (b) 2 h, (c) 4 h, respectively.

midline incision, and an occluding device described by Tomida et al. (1987) was implanted around the vessels. On the next day, the animals were subjected to total cerebral ischemia by the occlusion of carotid arteries for 20 min. After the procedure the device was released and a reperfusion period with different duration (2, 4 and 8 h) was allowed to the rats ($n = 6$ in each group).

Arterial blood pressure, and acid–base and blood gas parameters were measured before as well as 30 and 60 min after the intravenous injection in four subgroups of animals, i.e., in control and post-ischemic rats receiving either vehicle or 5 mg/kg DPPE. A catheter was inserted into the left femoral artery of rats under pentobarbital anesthesia, and mean arterial blood pressure was determined by a Statham P230 transducer (Statham Instruments, Los Angeles, CA, USA). Arterial blood chemistry parameters (pH, partial CO_2 tension, partial O_2 tension) were determined by the standard Astrup method using an ABL-330 equipment (Radiometer, Copenhagen, Denmark). Hematocrit was also determined at 60 min in arterial blood samples.

2.2. Blood–brain barrier permeability measurements

The development of vasogenic brain edema, as the extravasation of two intravascular permeability tracers: sodium fluorescein (MW = 376) and Evan's blue-labeled albumin (MW = 67,000), was measured as it was described in details (Ábrahám et al., 1996). The animals were given a solution of both dyes (2%, 5 ml/kg) in an intravenous injection 30 min before the end of the experiments. Then blood samples were taken and rats were perfused with 200 ml/kg isotonic saline. Serum as well as tissue samples from four brain regions (cerebral cortex, hippocampus, striatum, cerebellum) were homogenized in 1.0 ml of cold 15% trichloroacetic acid and centrifuged with $10{,}000 \times g$ for 10 min. The concentration of tracers was measured in supernatants by a Hitachi F2000 fluorimeter (Tokyo, Japan), the absorbency of Evan's blue at 620 nm, while the emission of sodium fluorescein at 525 nm after excitation at 440 nm. Blood–brain barrier permeability for a tracer was expressed as brain tissue concentration divided by serum concentration.

2.3. Drugs and chemicals

N,*N*-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine (DPPE, MW = 399.5) was synthesized by Dr. Ferenc Hudecz (Peptide Research Group, Department of Organic Chemistry, Eötvös Loránd University, Budapest, Hungary). All other drugs and chemicals used in the study were purchased from Sigma (St. Louis, MO, USA).

2.4. Statistical analysis

The values measured in different groups were compared using the analysis of variance (clinical data) or the Kruskal–Wallis one-way analysis of variance on ranks followed by the Student–Newman–Keuls test (permeability measurements). Changes were considered statistically significant at $P < 0.05$.

3. Results

3.1. Clinical parameters

Slow intravenous administration of 1 or 5 mg/kg DPPE was not followed by any major behavioral or postural change in male Wistar rats. However, the dose of 20 mg/kg proved to be toxic. It resulted in rapid elevation of muscle tone: extension in the forelegs and flexion in the hindlegs with immobility in the first 10–20 min. Gradual recovery was seen in five of six animals, while one rat died.

There was no significant change in mean arterial blood pressure between the four groups examined, namely in vehicle-, and 5 mg/kg DPPE-treated healthy rats, and in vehicle-, and DPPE-treated post-ischemic animals within 1 h of intravenous injection (Table 1). Similarly, arterial blood chemistry parameters (pH, pCO_2, pO_2, hematocrit) did not differ significantly in the above-mentioned animal groups (Table 1).

3.2. Effects of DPPE on the blood–brain barrier permeability in control rats

DPPE treatment, in an intravenous dose of 5 mg/kg, significantly ($P < 0.05$) increased the extravasation of

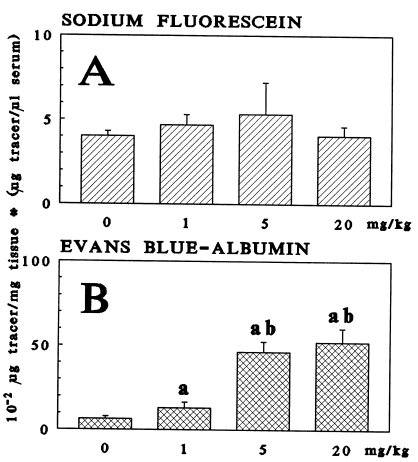

Fig. 2. Dose-dependent changes induced by DPPE in the blood–brain barrier permeability in parietal cortex of rats. Permeability for sodium fluorescein (A) and Evan's blue-albumin (B) was determined 4 h after the intravenous administration of 0, 1, 5, or 20 mg/kg DPPE. Extravasations were expressed as 10^{-2} μg tracer/mg brain tissue × (μg tracer/μl serum)$^{-1}$ for both dyes, each value presented is a mean ± S.E.M., $n = 5$ in 20 mg/kg DPPE group, while $n = 6$ in other animal groups. Letters indicate significant differences ($P < 0.05$) compared to values measured after following treatments: (a) 0 mg/kg; (b) 1 mg/kg, respectively.

sodium fluorescein in all regions but cerebral cortex 2 h after the injection (Fig. 1A,C,E,G). No significant change compared to the values measured in control rats was found later, except for a decrease in parietal cortex 8 h after the DPPE injection (Fig. 1A). However, a 5- to 12-fold increase in Evan's blue-labeled albumin extravasation developed 2 h after the DPPE administration in each brain region examined (Fig. 1B,D,F,H). Though albumin permeability remained significantly higher than control values, a further increase was seen in parietal cortex (Fig. 1B), while a tendency to decrease was measured in striatum (Fig. 1F) and cerebellum (Fig. 1H).

In parietal cortex, though DPPE in doses applied did not result in significant change in sodium fluorescein permeability at 4 h (Fig. 2A), a dose-dependent increase in albumin extravasation was seen (Fig. 2B). Similar tendencies were seen in the other three brain regions examined 4 h after the intravenous administration of 1, 5, and 20 mg/kg DPPE (data not shown).

3.3. Combined effects of cerebral ischemia–reperfusion and DPPE treatment on the blood–brain barrier permeability

Ischemia–reperfusion caused few significant alterations in the permeability of sodium fluorescein (Fig. 3A,C,E,G), namely an increase in parietal cortex at 4 h (Fig. 3A), and in striatum at 2 h (Fig. 3E). Compared to the values measured in vehicle-treated animals, 5 mg/kg DPPE administration resulted in the following changes in sodium fluorescein extravasation in the brain of post-ischemic animals. An increase was seen at 2 h in hippocampus and cerebellum (Fig. 3C–G), at 8 h in striatum and cerebellum (Fig. 3C–G), but a decrease developed in parietal cortex at 4 h (Fig. 3A).

Cerebral ischemia–reperfusion significantly ($P < 0.05$) increased the blood–brain barrier permeability for Evan's blue-bound albumin in each region examined (Fig. 3B,D,F,H). Albumin extravasation was also highly ele-

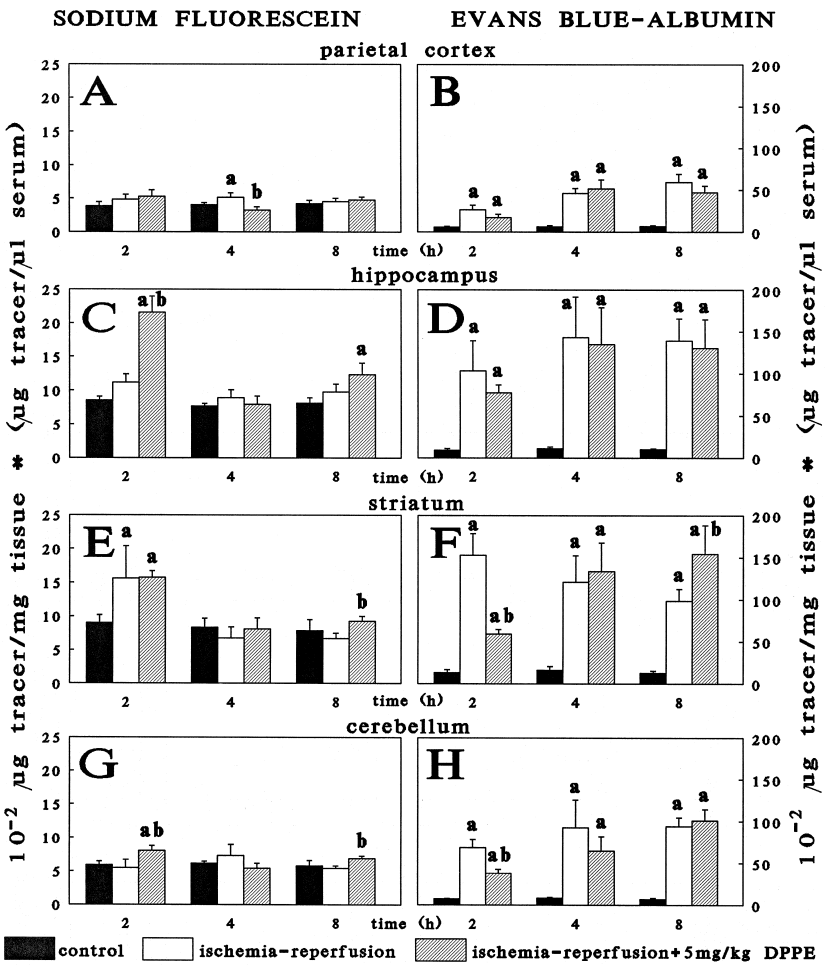

Fig. 3. Effects of cerebral ischaemia–reperfusion and intravenous DPPE administration on the blood–brain barrier permeability in the rat. Permeability for sodium fluorescein (A,C,E,G) and Evan's blue-albumin (B,D,F,H) was determined in parietal cortex (A,B), hippocampus (C,D), striatum (E,F), and cerebellum (G,H) of animal groups with the following treatments: control (filled bars), cerebral ischemia–reperfusion (open bars), and ischemia–reperfusion combined with DPPE administration (hatched bars). Extravasations were expressed as 10^{-2} µg tracer/mg brain tissue $\times$ (µg tracer/µl serum)$^{-1}$ for both dyes each value presented is a mean ± S.E.M., $n = 6$. Letters indicate significant differences ($P < 0.05$) compared to values measured at the same time-point, in the same brain region of the rats in: (a) control group; (b) ischemia–reperfusion group, respectively.

vated in DPPE-treated post-ischemic rats compared to the values measured in control animals (Fig. 3B,D,F,H). However, DPPE resulted in some time-dependent changes compared to data found in the brain of vehicle-treated rats underwent ischemia–reperfusion: albumin permeability was decreased at 2 h in striatum and cerebellum, but increased at 8 h in striatum (Fig. 3F–H).

4. Discussion

This report is the first to describe DPPE, a histamine Hic binding site antagonist, to induce a dose-dependent increase in the blood–brain barrier permeability in the rat. Moreover, DPPE also had some toxic side effects in a dose of 20 mg/kg. Cerebral ischemia induced by four-vessel occlusion and the consequent reperfusion resulted in a highly significant increase in the permeability for albumin, but only in minor changes in sodium fluorescein flux. DPPE, in a dose of 5 mg/kg, failed to prevent the blood–brain barrier opening induced by brain ischemia–reperfusion. Increased cerebrovascular permeability found after intravenous DPPE treatment suggest that intracellular histamine, as a second messenger, may have been involved in the maintenance of the blood–brain barrier characteristics.

4.1. Effect of DPPE on clinical parameters

In the present study, a single intravenous dose of 1 or 5 mg/kg DPPE did not result in changes in the behavior or some clinical parameters in male Wistar rats. Similarly, chronic subcutaneous injection of 1 or 4 mg/kg DPPE suggested to be non-toxic because it did not affect normal body weight gain in rats (Norrby, 1995). However, an intravenous dose of 20 mg/kg caused neurological symptoms and mortality in our experiments. In concordance with our data, subcutaneous injection of 20 mg/kg DPPE potentiated drug-induced seizures in male mice of BKTO strain (Sturman et al., 1994). We assume that DPPE could prevent the anticonvulsive effect of histamine, which was described to be mediated by histamine H_1 receptors in pentetrazole-induced seizures (Scherkl et al., 1991). In contrast with the DPPE toxicity shown, Cramer and Toorop (1998) reported on a significant protection by intraperitoneally administered 30 mg/kg DPPE against cerebral infarction without any side effects in male mice of NMRI strain. An explanation for this surprising observation can be that differences between inbred strains of mice define the threshold for convulsant-induced seizures (Van Buskirk and McGaugh, 1976), determine the susceptibility to cerebral ischemia (Barone et al., 1993), or even alter the histamine-induced changes in cerebrovascular permeability (Yong et al., 1994).

Since DPPE potentiates chemotherapy cytotoxicity to malignant cells (Brandes et al., 1991), it is used in combination with other drugs in clinical studies. Its beneficial effect was described in the treatment of patients suffering from refractory cancer of various origin (Brandes et al., 1994), unresponsive prostate cancer (Brandes et al., 1995), and metastatic breast cancer (Brandes and Bracken, 1998). However, after the administration of the maximally tolerated dose of DPPE (6 mg/kg) as an intravenous infusion, acute treatment toxicity, e.g., nausea, vomiting, drowsiness, light-headedness, mild to moderate incoordination, ataxia, mild visual or auditory hallucinations; as well as delayed effects, such as tiredness, and mild nausea, were recorded in the clinical studies mentioned. Brandes et al. (1994) also reported about decreases in body temperature and heart rate, but no change in blood pressure in cancer patients treated with DPPE in combination with a single chemotherapy drug. The acute side effects correlated with the peak serum levels of DPPE in the patients. The possibility raised by clinical observations that DPPE might modify the blood–brain barrier functioning is supported by our present findings.

4.2. Time- and dose-dependent blood–brain barrier permeability changes induced by intravenous DPPE administration

In the present study, histamine Hic receptor antagonist DPPE caused seemingly paradox changes in the cerebrovascular permeability: highly elevated extravasation of albumin, but moderate changes in the permeability for a small molecular weight tracer. However, these tracers cross cerebral endothelial cells in different ways: albumin by transcytosis (Broadwell and Banks, 1993), while sodium fluorescein mainly through the paracellular route (Thompson et al., 1994). The pattern found after DPPE treatment with a predominance of increased transendothelial macromolecular transport resembles to the barrier permeability changes seen after the administration of exogenous histamine. Intracarotid histamine challenge induced high pinocytotic activity in adult rats (Dux and Joó, 1982), and a substantial increase in permeability for albumin, but a lesser change in that for sodium fluorescein, in newborn pigs (Németh et al., 1997, 1999). In a recent in vitro study, histamine increased the transcellular passage of albumin through the brain endothelial monolayer, but the permeability for the markers of paracellular route, remained unchanged (Deli et al., 1995b). Considering these data and our present findings one can assume that histamine Hic binding site antagonist DPPE may have histamine-like effects on the blood–brain barrier permeability in rats.

Histamine increases the blood–brain barrier permeability by histamine H_2 receptor-dependent ways, mainly by the activation of adenylate cyclase enzyme (see, for review, Wahl et al., 1988; Edvinsson et al., 1993; Joó, 1993). Histamine, similar to cyclic adenosine 3′5′-monophosphate (cAMP) or cyclic guanosine 3′5′-monophosphate (cGMP), induced the formation of pinocytotic vesi-

cles in cerebral endothelium in vivo (see, for review, Joó, 1996). In our in vitro experiments, however, cAMP treatment resulted in a rapid decrease in paracellular permeability of brain endothelial cells (Deli et al., 1995a), while histamine administration elevated the albumin transport, but did not alter the permeability of tight junction markers (Deli et al., 1995b). Histamine might affect the blood–brain barrier permeability by histamine H_1 receptor dependent mechanisms, too (Joó, 1996). In cultured cerebral endothelial cells, histamine could stimulate phospholipase C (Purkiss et al., 1994), and elevate the intracellular [Ca^{2+}] concentrations in vitro (Revest et al., 1991), suggesting a phosphoinositol-mediated process. Histamine could also increase the cerebrovascular permeability through a nitric oxide-mediated activation of guanylate cyclase (Mayhan, 1996). In peripheral endothelial cells, a similar mechanism involving consequent phospholipase C activation, Ca^{2+}-release from intracellular stores, induction of nitric oxide synthase, stimulation of guanylate cyclase, and formation of cGMP is proven to be responsible for the histamine-induced increase in albumin permeability (Yuan et al., 1993). In addition, a linear relationship was found between the activity of acid phosphatase enzymes and increased blood–brain barrier permeability (Németh et al., 1997), and a role for the lysosomal and cytosolic acid phosphatase isoforms was assumed in the regulation of the permeability (Németh et al., 1999). Moreover, it is supposed that histamine can also increase the albumin permeability by a rearrangement of endothelial actin cytoskeleton in brain endothelial cells, similarly to that in peripheral ones (Rotrosen and Gallin, 1986; Baldwin and Thurston, 1995).

DPPE did not have a classical histamine H_1 and H_2 receptor antagonist effect on the permeability in our model which may indicate that DPPE is not acting through the histamine H_1 and H_2 receptors in the blood–brain barrier. A possible explanation for the findings is that histamine might have a dual role in cerebral microvessels: exogenous histamine increases the permeability through histamine H_1 and H_2 receptors which can be blocked by conventional histamine receptor antagonists, whereas intracellular histamine can maintain barrier properties through histamine Hic binding sites. This later function can be disrupted by DPPE, leading to a permeability increase similar to that seen after exogenous histamine administration. A dual role of endogenous histamine was originally suggested by Norrby (1995) on mast-cell mediated angiogenesis based upon studies with DPPE.

4.3. Effect of cerebral ischemia–reperfusion on permeability

According to the classical neuropathological views, the essential events in the development of vasogenic brain edema formation are increased blood–brain barrier permeability, enhancement of driving forces including a bulk flow into the interstitial space of the brain, and retention of fluid (Wahl et al., 1988). Surprisingly, during post-ischemic reperfusion the increase in the extravasation of Evan's blue-labelled albumin (MW = 67,000) was much higher in each region examined, than the change in the permeability of sodium fluorescein (MW = 376). Similar to our present and previous (Németh et al., 1998) observations, Todd et al. (1986) also reported no change in the cerebrovascular permeability for [^{14}C]α-aminoisobutyric acid (MW = 104) in the four-vessel occlusion model in male Sprague–Dawley rats. Dramatic increase in the permeability for albumin, but more moderate change for [^{3}H]sucrose (MW = 342), was also obtained on an in vitro reconstituted blood–brain barrier model after hypoxia (Plateel et al., 1997). Total cerebral ischemia–reperfusion resulted in a disproportionally huge increase in the permeability for albumin, a transendothelial marker, compared to that for the paracellular tracer sodium fluorescein, in adult rodents. No such a phenomenon was found with the same tracers in newborn pigs suffered from asphyxia (Ábrahám et al., 1999) or in those challenged by intracarotid tumor necrosis factor-α (Ábrahám et al., 1996; Megyeri et al., 1999). It may indicate that age-, species-, or stimulus-specific changes would be responsible for the selective activation of macromolecular transport in our model.

4.4. DPPE and blood–brain barrier: possible modes of action

It was suggested in previous animal studies that both histamine H_1 and H_2 receptor antagonists were beneficial in the prevention of ischemic brain edema (Gross et al., 1981; Dux et al., 1987; Edvinsson et al., 1993; Joó, 1993, 1996; Tósaki et al., 1994). In contrast to these antagonists, histamine Hic binding site antagonist DPPE could not prevent, although slightly modified, the development of post-ischemic brain edema. Moreover, DPPE induced an increase in the blood–brain barrier permeability for intravascular tracers in healthy adult rats. This observation supports that intracellular histamine may participate in the maintenance of the barrier properties, but other molecular mechanisms should also be taken into consideration. There is no data available that DPPE would exert a direct agonist effect on histamine H_1 and H_2 receptors of cerebral endothelium, moreover DPPE had a weak histamine H_1 receptor antagonist effect in other cell types (Brandes et al., 1990). Supposing that DPPE can increase the endothelial intracellular histamine concentration by blocking the histamine Hic binding sites, this change may result in higher rate of histamine efflux, thereby an indirect agonist effect on conventional histamine H_1 and H_2 receptors. However, it seems unlikely, because we could not find an increased cerebrovascular permeability in DPPE-treated post-ischemic rats compared to DPPE-treated control ones, although cerebral ischemia significantly increases brain histamine levels (Adachi et al., 1992; Kovács et al., 1995).

Emerging role for histamine as an intracellular second messenger has been established in the regulation of cellular processes in a wide variety of cell types (Brandes et al., 1990), but no information was published about the involvement of histamine Hic binding sites in the regulation of the blood–brain barrier properties. Recently, Karlstedt et al. (1999) have found a lack of histamine synthesis in immortalized RBE4 cells, and also provided evidence that the internalized histamine was distributed in the cytoplasm and nucleus of the endothelial cells. It raised the possibility that cerebral endothelial cells forming the blood–brain barrier possess histamine Hic receptors, and might suggest a role to be elucidated for these binding sites. However, a different histamine uptake mechanism was described in RBE4 cells (Karlstedt et al., 1999), than in cultured primary brain endothelial cells (Huszti et al., 1995, 1997). One cannot exclude that the regulation of histamine metabolism is different in primary cultures of brain capillary endothelial cells and immortalised cell lines, similarly to the alterations found in the eicosanoid and nitric oxide metabolisms (Kis et al., 1999).

On the other hand, the permeability of the blood–brain barrier is not regulated exclusively by the cerebral endothelial cells, but other cooperating cells, i.e., astrocytes, neurons, microglial cells, pericytes, smooth muscle cells, may also influence it (Joó, 1996). Blood–brain barrier functions can also be modulated by a variety of blood cells which are influenced by histamine, as a second messenger. These cells include lymphocytes, monocytes, neutrophil leukocytes (Falus and Merétey, 1992), and platelets (Brandes et al., 1990). Considering our in vivo results obtained on rats, one should not exclude that cellular elements other than brain endothelial cells are also responsible for the blood–brain barrier permeability changes found in DPPE-treated animals. We think the in vitro approach as an appropriate model to reveal the contribution of cerebral endothelium to permeability changes, and hopefully it would also provide some details about the molecular mechanisms implicated.

Favoring the view that DPPE might induce an increase in the blood–brain barrier permeability by the mediation of histamine Hic binding sites, some other modes of action should also be taken into consideration. First, a possible explanation for the DPPE-induced blood–brain barrier permeability changes could be if the histamine Hic binding site antagonist would interfere with the binding of growth-modulatory endogenous bioamines to the microsomal cytochrome *P*450 in brain endothelium. Brandes et al. (1998) claimed that intracellular histamine could bind to cytochrome *P*450 in liver microsomal fraction, and polyamines would allosterically compete for these sites. It was suggested that histamine would also bind potently to the nitrophorin, a heme protein, which could release nitric oxide in the salivary gland of an arthropod (Ribeiro and Walker, 1994). However, there is no proof that these mechanisms can take place in cerebral or even in peripheral endothelial cells. On the other hand, polyamines synthesized by ornithine decarboxylase can act as intracellular messengers and their role is established in the mediation of the blood–brain barrier breakdown (Koenig et al., 1983).

An alternative explanation for the permeability-inducing effect is that DPPE, as a ligand for the microsomal antiestrogen binding sites, can also exert its effect through that way (Brandes et al., 1990). In peripheral endothelia, tamoxifen and estradiol increased the expression of vascular endothelial growth factor (Hyder et al., 1996), a well-known inducer of angiogenesis and blood–brain barrier disruption (Wang et al., 1996). This mechanism may also serve as an explanation for the dual role suggested of endogenous histamine in the mast-cell mediated angiogenesis (Norrby, 1995). It was found in mesenteric vessels of rats, that specific histamine H_1 and H_2 receptor antagonists significantly suppressed, whereas DPPE treatment increased the rate of angiogenesis (Norrby, 1995). A third possibility is that the effect of DPPE can be connected with its affinity for the sigma receptor claimed by Cramer and Toorop (1998). There is no paper published about the effect of sigma receptor ligands on the blood–brain barrier, though these ligands were neuroprotective in cerebral ischemia (O'Neill et al., 1995). Another consideration can be that DPPE, which inhibits thromboxane production in platelets (Brandes et al., 1990), might also change the arachidonic acid metabolism in brain endothelium. A possible shift in the production of arachidonic acid metabolites may also contribute to the blood–brain barrier permeability changes, especially under post-ischemic conditions.

4.5. Conclusions

DPPE, a histamine Hic binding site antagonist, in doses used in the cancer therapy of human patients, induced an increase in the blood–brain barrier permeability in adult rats. The cellular and molecular mechanisms through which this effect is mediated are not yet established. Considering the therapeutical value of DPPE in the potentiation of the efficacy of chemotherapy, it would be worthwhile to reveal the effects of this drug on the blood–brain barrier permeability in patients enrolled into the ongoing clinical studies. Even if we suppose that DPPE treatment would cause similar increases in blood to brain flux in humans, it is difficult to predict the clinical relevancy of the findings: this effect can be harmful, negligible, neutral, or even beneficial, e.g., in the chemotherapy of brain tumors. Future investigations to determine the effects of histamine, as an intracellular messenger, on the regulation of the blood–brain barrier characteristics can be a novel and interesting research field with potential clinical benefits.

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