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Oleic acid reversibly opens the blood–brain barrier

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This study examined the effect of intracarotid oleic acid infusion on blood–brain barrier permeability Oleic acid was infused for 30 s at a rate of 6 ml/min into the right internal carotid artery at concentrations of 10^{-6}, 10^{-5}, 2×10^{-5} and 5×10^{-5} M Extensive Evans blue-albumin extravasation was observed 15 min after the administration of 2×10^{-5} M oleic acid The permeability surface area product for α-aminoisobutyric acid (AIB), determined 1–11 min following the infusion of oleic acid was increased 10-fold following infusion of 10^{-5} M oleic acid and 20-fold following the administration of 5×10^{-5} M oleate The blood–brain barrier opening to AIB proved to be reversible 80–90 min after the infusion of 2×10^{-5} M oleic acid The possible mechanisms of the oleic acid effect are discussed

INTRODUCTION

The capillaries in brain are formed by a specialized endothelium whose function is to regulate the movement of solutes between blood and brain The endothelial cells in brain capillaries are sealed together by continuous tight junctions and contain few pincytotic vesicles These properties limit the free exchange of water-soluble non-electrolytes, electrolytes and proteins from blood to brain and are responsible for formation of a blood–brain barrier (BBB)[6,25,27]

Since brain uptake of many neuropharmacologic agents is limited by the BBB, a method for reversibly increasing cerebrovascular permeability could enhance treatment of a variety of neurologic diseases Osmotic BBB opening has been characterized quantitatively and shown to be reproducible and reversible[23] It is believed to increase BBB permeability by disrupting intracellular tight junctions[4] Clinically, this procedure has been shown to increase the delivery of chemotherapeutic agents to the brain[17], but its use for treatment of patients is not yet widely accepted[5,7]

Alternative approaches to increasing BBB permeability would be to alter endothelial cell membrane integrity or to activate intracellular second messenger systems that regulate BBB permeability In recent years, there have been many studies on the biological effects of the fatty acids[3,11,13,14,18,20,29] and there is growing evidence that they modify membrane structure and function[26] Low concentrations of several unsaturated fatty acids such as oleic, linoleic and linolenic acid exert a stabilizing effect on the erythrocyte membrane, whereas higher concentrations of these same agents cause membrane lysis[26] Furthermore, protein kinase C, an enzyme which can be detected in brain capillaries[22] and plays an important role in the regulation of cellular functions[16], is activated by unsaturated fatty acids[13,14] Liberation of various free fatty acids from brain tissue has been observed during injuries that are associated with BBB opening, such as ischemia[15] and cold injury[1] Much attention has been focused on the role of arachidonic acid and less on the other unsaturated fatty acids as possible contributing agents in the BBB destruction and brain edema formation that accompanies these injuries[3,29] In this study, we determined the effect of an intracarotid infusion of sodium oleate on BBB permeability

MATERIALS AND METHODS

Adult male Sprague–Dawley rats, weighing between 350 and 400 g were anesthetized by intramuscular injection of 50 mg/kg ketamine hydrochloride and 10 mg/kg xylazine Polyethylene catheters (PE-50) filled with heparinized saline were inserted into the right femoral vein for the administration of the tracers and into the femoral arteries for continuous pressure recording and blood sampling After exposure of the right carotid artery, the occipital, superior thyroid and pterygopalatine arteries were ligated, and the right external carotid artery was catheterized for retrograde infusion

Oleic acid sodium salt (Sigma, St Louis, MO) was dissolved in 20% ethanol and diluted in saline to concentrations of 10^{-6}, 10^{-5}, 2×10^{-5} and 5×10^{-5} M The stock solutions were prepared at various concentrations so that the final amount of ethanol was the same in each dilution (0 004% ethanol) The osmolality of the

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solutions was between 270 and 290 mOsm and the pH was adjusted to 7 4 with sodium hydroxide The oleic acid was infused into the right internal carotid artery at a rate of 6 ml/min for 30 s This rate of infusion visibly cleared the internal carotid artery of blood The control rats were infused with saline, pH 7 4, containing the same amount of ethanol as the oleic acid solutions

A 2 ml/kg b wt dose of Evans blue (2% g/vol) solution was injected intravenously in a group of 5 rats, 30 min before the intracarotid infusion of 2×10^{-5} M oleic acid The animals were decapitated 15 min after the carotid infusion and brains were removed, sliced and examined visually for Evans blue extravasation This quantity of dye binds almost completely to plasma albumin and as a dye–protein complex is a qualitative marker of BBB integrity[24]

The permeability of cerebral blood vessels to [^{3}H]aminoisobutyric acid (AIB)[2] was examined by a modification of the method of Ohno et al [19] as described previously[12] A 0 1 ml saline bolus containing 45 μCi [^{3}H]AIB (DuPont/NEN, Boston, MA) was injected into the femoral vein and allowed to circulate for 10 min During this time, blood was continuously withdrawn from a femoral artery into polyethylene tubing At the end of the 10 min circulation time, a blood sample was taken from the other femoral artery cannula to determine the final plasma isotope concentrations and hematocrit The animal was then decapitated and the brain removed The brain was dissected into right and left halves and then the anterior and posterior cortex, hippocampus, basal ganglia, cerebellar hemispheres and brainstem were separated These tissues were dissolved in Protosol (DuPont/NEN, Boston, MA) Aliquots of plasma from the terminal blood sample were dissolved in Protosol and aliquots of the continuously withdrawn blood sample were dissolved in Protosol ethanol (2 1 v/v) Samples were counted in a two-channel liquid scintillation counter

For [^{3}H]AIB, brain uptake can be expressed as the permeability surface area (PS) product according to

$$PS = C_{ev}/\int C_a \, dt$$

where C_{ev} is the amount of extravascular tracer in the brain and C_a is the arterial plasma concentration The integral of C_a with time was calculated from the amount of isotope in the continuously withdrawn arterial blood sample (C_o), the withdrawal rate (F_o) and the arterial hematocrit (Hct)

$$\int C_a \, dt = C_o(1 - Hct)/F_o$$

The amount of extravascular tracer in the brain, C_{ev}, was calculated from the total brain radioactivity minus the terminal plasma concentration of [^{3}H]AIB multiplied by the plasma volume For the determination of the regional plasma volumes, 15 μCi [^{14}C]dextran (DuPont/NEN, Boston, MA) was injected via the femoral vein 3 min before the animal was decapitated The plasma volume of the brain (ml/g) is equal to the ratio of the ^{14}C-counts in brain (per g) and plasma (per ml)

The [^{3}H]AIB was given 1 min after completion of the carotid infusion of 10^{-6}, 10^{-5}, 2×10^{-5}, 5×10^{-5} M oleic acid One group of rats that received 2×10^{-5} M oleic acid was injected with [^{3}H]AIB 80 min after the carotid infusion to examine the reversibility of the blood–brain barrier opening Five rats were used in the control and each experimental group The analysis of variance employing Dunnet's test was used for the statistical comparisons of experimental and control groups

RESULTS

The physiological parameters are summarized in Table I There were no differences among the groups in the mean arterial blood pressure (MABP), arterial pH, pCO_2, pO_2 or hematocrit Animals survived the higher doses of oleic acid for up to 2 h without obvious detrimental effects

The most pronounced Evans blue extravasation following infusion at 2×10^{-5} M oleic acid (Fig 1) was observed in the right cortical areas and hippocampus and the left parasagittal cortical area Occasionally Evans blue staining appeared in the right cerebellar hemisphere Within a given brain region, the pattern of Evans blue extravasation showed a somewhat heterogeneous distribution

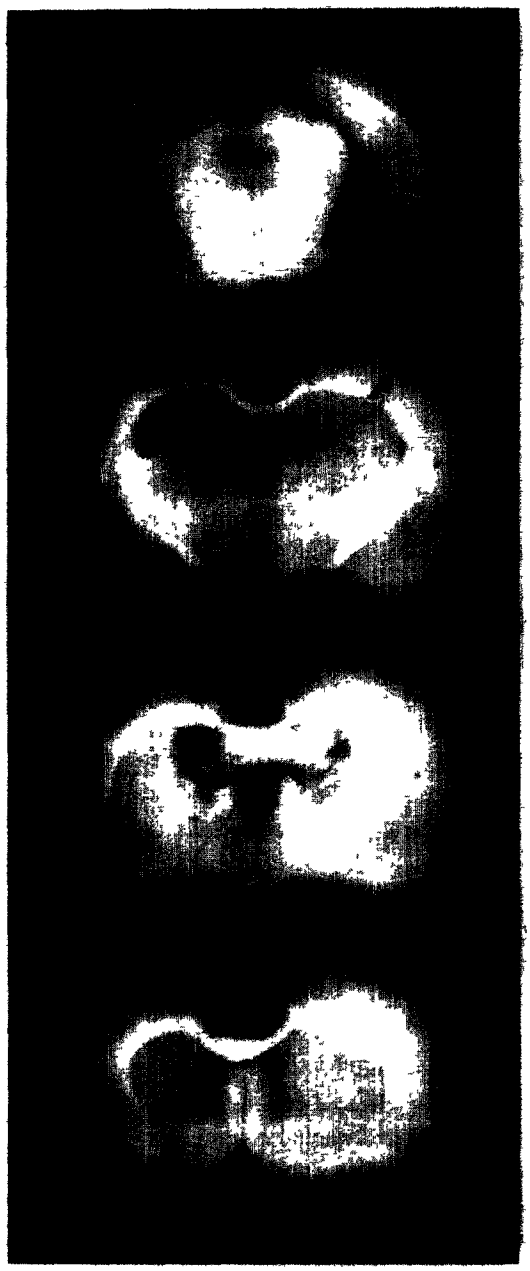

Fig 1 Evans blue extravasation 15 min after right sided intracarotid infusion of 2×10^{-5} M oleic acid The appearance of the Evans blue staining is heterogeneous, with pronounced staining in the right cortical and hippocampal areas and the left parasagittal cortical areas

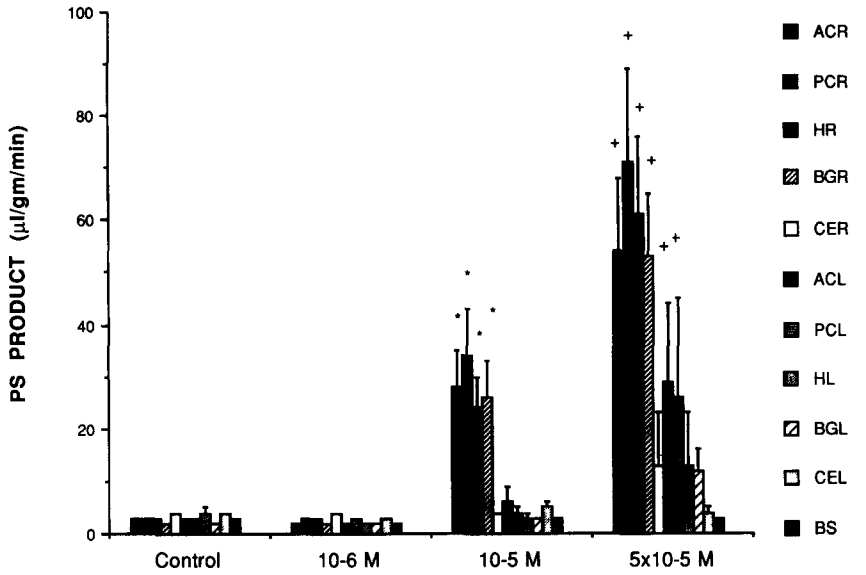

Fig 2 Effect of different concentrations of oleic acid on BBB permeability to AIB Between 1 and 11 min following the infusion of 10^{-5} M oleic acid there was a 10-fold increase in the AIB PS product of the infused hemisphere After 5×10^{-5} M oleate, a further increase was observed ACR, PCR, HR, BGR, CER right sided anterior and posterior cortex, hippocampus, basal ganglia and cerebellum, respectively ACL, PCL, HL, BGL, CEL similar but on the left side BS brainstem Data points represent the means ± S E M of 5 rats in each group $^{*}P < 0\ 05$, $^{**}P < 0\ 01$ compared with the controls and 10^{-6} M oleate $^{+}P < 0\ 05$, $^{++}P < 0\ 01$ compared with 10^{-5} M oleate

The [^{3}H]AIB PS product varied between 2 and 4 μl/g/min in the brain regions of the saline-perfused control rats (Fig 2) These values are similar to those reported previously for normal rat brain[12] and indicate that the sudden perfusion of the rat brain with saline does not, by itself, cause the BBB to open No change was observed after infusion of 10^{-6} M oleic acid, but a significant, 10-fold increase was measured in the areas of the right hemisphere after the administration of 10^{-5} M oleic acid (Fig 2) A further two-fold increase in the [^{3}H]AIB PS product in the right sided brain regions and a significant increase in the right cerebellar hemisphere

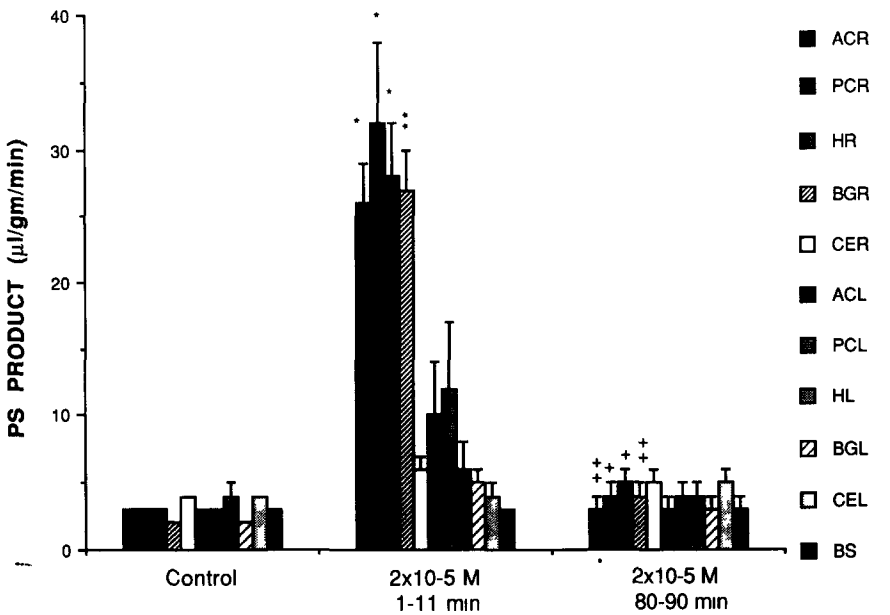

Fig 3 Reversibility of the changes in the blood–brain barrier permeability to AIB Immediately after the infusion of 2×10^{-5} M oleic acid there was a large increase in the AIB PS product, however, 80–90 min following the infusion the values returned to the control level Data points represent the means ± S E M of 5 rats in each group $^{*}P < 0\ 05$ compared with the controls $^{+}P < 0\ 05$ compared with 2×10^{-5} M 1–11 min group There was no significant difference between the control and the 2×10^{-5} M 80–90 min groups

TABLE I

Physiological parameters

Values are means ± S E M of 5 animals

	Controls	*10^{-6} M*	*10^{-5} M*	*2×10^{-5} M*	*5×10^{-5} M*	*2×10^{-5} M + 80 min*
MABP (mm Hg)	97 ± 6	87 ± 5	90 ± 4	87 ± 5	91 ± 5	92 ± 6
Temp (°C)	36 5 ± 0 1	36 7 ± 0 2	36 5 ± 0 1	36 7 ± 0 1	36 5 ± 0 1	36 8 ± 0 1
Arterial pH	7 37 ± 0 01	7 35 ± 0 01	7 35 ± 0 02	7 36 ± 0 02	7 31 ± 0 02	7 34 ± 0 01
p_aCO_2 (mm Hg)	45 ± 1	46 ± 4	38 ± 5	43 ± 3	41 ± 7	46 ± 2
p_aO_2 (mm Hg)	77 ± 3	73 ± 3	72 ± 7	84 ± 5	72 ± 5	80 ± 5
Hematocrit (%)	36 ± 1	37 ± 1	33 ± 2	36 ± 2	34 ± 4	36 ± 2

and the left cortical areas was observed after infusion of 5×10^{-5} M oleic acid (Fig 2)

The reversibility of the oleic acid-induced BBB opening to [^{3}H]AIB was examined 1–11 and 80–90 min following the infusion of 2×10^{-5} M oleic acid This dose caused an initial increase in the AIB permeability of the same magnitude as a dose of 10^{-5} M, however, by 80–90 min later, the permeability returned to the control values (Fig 3)

A higher [^{14}C]dextran space was measured in the right sided cerebral regions parallel with the increased AIB permeability, an increase that was statistically significant in the animals perfused with 5×10^{-5} M oleic acid (Table II) This increase most likely represents extravasation of [^{14}C]dextran as a result of the extensive BBB opening rather than a true increase in plasma volume Consequently, the values of AIB permeability at the higher doses of oleic acid are probably underestimates of the true permeability

DISCUSSION

Oleic acid increases BBB permeability in a dose-dependent, reversible manner The increase in the AIB PS product in the cortical regions of the left hemisphere and right cerebellar hemisphere can be explained as a result of cross perfusion to the contralateral hemisphere and ipsilateral cerebellar areas as indicated by the pattern of the extravasated Evans blue The heterogeneous Evans blue staining indicates an uneven distribution of areas with increased albumin permeability As the flow rate of the infusion was high, no streaming phenomena can be expected[28], and the cause of the uneven appearance of the albumin staining remains to be explained

Since the BBB is created by the plasma membrane and tight junctions of the endothelial cells, the change in BBB permeability caused by oleic acid might be explained as a lipid–membrane interaction The experiments of Raz and Livne[26] demonstrated that the osmotic fragility of erythrocytes is affected by oleic acid Low concentrations

TABLE II

Effect of oleic acid on [^{14}C]dextran space

Values are means ± S E M of 5 animals and expressed in units of μl/g ACR, PCR, HR, BGR, CER right sided anterior and posterior cortex, hippocampus, basal ganglia and cerebellum, respectively ACL, PCL, HL, BGL, CEL similar but on the left side BS brainstem

	Controls	*10^{-6} M*	*10^{-5} M*	*2×10^{-5} M*	*5×10^{-5} M*	*2×10^{-5} M + 80–90 min*
ACR	6 9 ± 0 5	6 9 ± 0 5	13 0 ± 2 0	11 9 ± 1 1	27 2 ± 7 6*	8 3 ± 1 4
PCR	7 0 ± 0 5	7 0 ± 0 5	14 2 ± 2 1	14 7 ± 0 7	30 7 ± 7 7*	7 4 ± 0 9
HR	7 0 ± 0 8	8 2 ± 1 0	13 2 ± 1 3	14 6 ± 1 3	35 2 ± 10 1*	7 6 ± 0 6
BGR	7 3 ± 0 9	8 0 ± 0 4	12 3 ± 1 2	12 6 ± 0 3	27 9 ± 7 0*	7 6 ± 0 3
CER	12 4 ± 1 4	12 1 ± 2 1	13 1 ± 1 1	13 4 ± 1 3	17 3 ± 2 6**	9 8 ± 0 6
ACL	7 3 ± 0 5	7 5 ± 0 2	8 3 ± 0 9	9 0 ± 0 6	17 3 ± 5 6**	6 6 ± 0 2
PCL	7 8 ± 0 7	8 7 ± 0 6	9 4 ± 1 1	9 2 ± 0 6	13 3 ± 4 5	8 5 ± 0 7
HL	8 7 ± 0 6	8 1 ± 1 0	9 4 ± 1 3	9 1 ± 1 5	10 4 ± 3 0	6 8 ± 0 4
BGL	7 1 ± 0 4	8 4 ± 0 7	8 9 ± 0 4	8 3 ± 0 6	9 9 ± 2 5	7 7 ± 0 5
CEL	11 8 ± 0 8	13 2 ± 1 2	12 5 ± 1 6	12 4 ± 0 9	13 4 ± 0 4	10 7 ± 0 6
BS	9 4 ± 0 9	9 0 ± 0 6	11 1 ± 0 4	14 8 ± 4 0	9 7 ± 0 7	8 9 ± 0 4

*$P < 0$ 01 compared with the controls and the 10^{-5} M group

**$P < 0$ 05 compared with the controls and the 10^{-5} M group

reduced the proportion of cells that were lysed while higher oleic acid concentrations (10^{-5} M and higher) increased their susceptibility to osmotic shock In our experiments the same threshold concentration range of oleic acid was found to be damaging to the BBB Oleic acid is an amphiphilic substance which can induce major changes in membrane function[10] At low concentrations, as a monomer, it inserts itself into the lipid membrane, changing the physical properties and physiological function of the membrane in a way that appears as a membrane-stabilizing effect[10] At high concentrations, however, amphiphilic monomers aggregate into micelles which have the ability to incorporate membrane lipids into their structure The incorporation of high concentrations of amphiphiles into membranes physically disrupts the lipid bilayer and, consequently, destroys the integrity of biological membranes[10] If an oleic acid–membrane interaction is responsible for the increased permeability of the BBB, it is surprising that the process is so rapidly reversible

Oleic acid is also known to be a potent activator of protein kinase C[13,14], a key enzyme in mediating transmembrane signaling[16] The presence of protein kinases in the brain capillaries has been shown previously[22] and modulation of the kinetics of protein phosphorylation by second messengers may contribute to the regulation of the BBB permeability[21] Although there is only indirect evidence that protein kinase C may play a role in the regulation of the BBB transport processes[9], the possibility exists that oleic acid may affect the permeability of the brain vascular endothelial cells by changing protein kinase C-induced protein phosphorylation The rapid reversibility of the oleic acid-induced BBB opening supports the view that a reversible biochemical process is the underlying event

Recent studies[11 18 20] have revealed yet other biological effects that are exerted by oleic acid that could influence the permeability of the BBB Reduced synaptosomal Na^+,K^+-ATPase activity[20] and inhibition of the mitochondrial oxidative phosphorylation[19] were observed after treatment with oleic acid The release of reactive oxygen metabolites may also be involved in barrier destruction as oleic acid-induced pulmonary edema can be attenuated by pretreatment with catalase and superoxide dismutase[11]

Oleic acid in cortical superfusion experiments performed by Unterberg et al [29] was found to be without effect on the BBB of pial vessels Given intracerebrally it did not induce changes in brain water and cation levels[3] The discrepancy between our results and those of others may be explained by the different route of administration of the oleic acid Increased oleic acid concentrations were measured in the plasma and interstitially drained brain edema fluid in cats exposed to cold injury[1] and in the brain tissue following ischemia[8,15 30] Our results indicate that the elevated oleic acid concentration might contribute to the disturbance of BBB permeability observed in these experimental models However, binding of oleic acid to albumin would normally reduce the concentration of free oleic acid in the plasma by a considerable amount In our studies, the intracarotid infusion rate was high enough to completely clear the blood from the carotid circulation on the side of the infusion Thus, there was no binding of oleic acid to albumin during the infusion and the BBB was exposed to the stated concentrations of free oleic acid We are unable to predict whether albumin-bound oleic acid would have a similar effect

In conclusion, intracarotid infusion of oleic acid increases BBB permeability in a dose-dependent and reversible fashion The procedure is well tolerated in the short term, however, neither long-term toxicity nor localized effects leading to cellular injury, brain edema, and metabolic disturbances have been examined It is unclear at the present time whether the BBB opening induced by oleic acid results from a non-specific lipid-membrane interaction or the activation of a specific biochemical pathway Further studies are warranted since this procedure may provide a useful clinical approach to modification of BBB permeability

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