intercurrent infection in patients with rheumatoid arthritis. Arthrit. Rheum. 32: 347–351

Pos, O., Oostendorp, R. A., van der Stelt, M. E., Scheper, R. J., Van Dijk, W. (1990) Con A-nonreactive human alpha 1-acid glycoprotein (AGP) is more effective in modulation of lymphocyte proliferation than Con A-reactive AGP serum variants. Inflammation 14: 133–141

Ricca, G. A., McLean, J. W., Taylor, J. M. (1982) Kinetics of induction of α_1-acid glycoprotein. Ann. NY Acad. Sci. 389: 88–105

Routledge, P. A. (1989) Clinical relevance of alpha 1 acid glycoprotein in health and disease. Prog. Clin. Biol. Res. 300: 185–198

Sagawa, J., Miyagi, T., Tsuiki, S. (1988) Membrane-associated sialidase of rat liver and its decrease in hepatomas. Jpn. J. Cancer Res. (Gann) 79: 69–73

Schmid, K. (1975) Alpha-1-acid glycoprotein. In: Putnam, F. W. (ed.) The Plasma Proteins vol. 1. Academic Press, New York, pp 183–228

Serbource-Goguel Seta, N., Durand, G., Corbic, M., Agneray, J., Feger, J. (1986) Alterations in relative proportions of microheterogenous forms of human alpha-1-acid glycoprotein in liver disease. J. Hepatol. 2: 245–252

Urban, J., Chan, D., Schreiber, G. (1979) A rat serum glycoprotein whose synthesis rate increased greatly during inflammation. J. Biol. Chem. 254: 10565–10568

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Ageing influences haloperidol-induced changes in the permeability of the blood-brain barrier in the rat

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Abstract—The effect of the dopaminergic antagonist haloperidol on the permeability of the blood-brain barier (BBB) to [^{14}C]α-aminoisobutyric acid was studied in 10–12- and 28–30-week old rats. Following the intraperitoneal injection of haloperidol (1 mg kg^{-1}), an increase in the permeability of the BBB, with respect to younger animals, was observed within the occipital cortex, striatum, hippocampus and hypothalamus in the older rats. No correlation was found between haloperidol-induced changes and age-related differences in the permeability of the BBB. Such age-associated increase in the vulnerability of the BBB when challenged with haloperidol might be related to a deterioration of the dopaminergic control of cerebrovascular permeability.

The blood-brain barrier (BBB) acts as a selectively permeable membrane able to maintain normal ionic differences between the blood and brain extracellular fluid (Rapoport 1976; Pardridge 1987, 1988); clearly the structural and functional integrity of the BBB is essential to normal neurological function. Apart from several known mechanisms by which the BBB breaks down, other circumstances can alter the non-specific permeability of the BBB, in the absence of ultrastructurally visible tearing of endothelial membranes and of gross changes or degeneration processes of the vascular patterns of the brain (Rapoport et al 1972; Petito et al 1982; Ellison et al 1986). In particular, we reported ageing-related modifications in the permeability of the BBB, probably reflecting alterations in brain neurochemical systems (Saija et al 1990a). In addition, there is wide evidence that centrally-acting drugs, such as amphetamine (Sankar et al 1983), arecoline (Saija et al 1990b), pentobarbitone and ketamine (Saija et al 1989), can induce modifications in the normal functioning of the BBB through different and perhaps concomitant mechanisms (direct action at the level of receptors present in the cerebral capillary endothelium and subserving vasoregulatory responses, changes in the neurogenic component controlling BBB permeability, alterations in the release of vasoactive substances). Furthermore, few data are reported concerning ageing-related changes in the effects of centrally acting drugs on the permeability of the BBB. The present study was undertaken to compare the changes in the functional activity of the BBB induced by the dopaminergic antagonist haloperidol in young and aged rats. Determination of the permeability of the BBB involved the use of a small molecular weight radiolabelled amino acid, [^{14}C]α-aminoisobutyric acid ([^{14}C]AIB); this tracer is only minimally transported across normal cerebral microvessels and is actively transported into the cells, so that the isotope is trapped in viable parenchymal cells (Blasberg et al 1980, 1983a, b; Gross et al 1982; Picozzi et al 1985; Saija et al 1990b), allowing the precise localization and quantitative expression of BBB changes.

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Materials and methods

Animal preparation. Male Wistar rats, 10–12-weeks old, 220–240 g, and 28–30-weeks old, 450–500 g, were kept under standardized conditions, with free access to food and water, and a 12 h light/dark period (light on: 0600 h).

On the day of the experiment, the animals were anaesthetized with pentobarbitone sodium (54 mg kg^{-1}, i.p.) and spontaneously breathed room air. Short PE20 polyethylene catheters filled with 100 int. units mL^{-1} heparin in 0·9% NaCl (saline) were inserted into the left femoral vein and artery for blood sampling and administration of tracers. Before isotope injection and periodically during the experiment, arterial blood samples were withdrawn for measurements of arterial blood gases and pH. Body temperature was maintained at 37°C by external heating. All experiments were carried out in the morning, between 0900 and 1100 h.

BBB permeability. Details of techniques for determination of the permeability of the BBB have been presented previously (Saija et al 1989, 1990a, b). A bolus of 15–25 μCi [^{14}C]AIB was injected intravenously. Blood was collected periodically from the femoral artery until the rat was killed by decapitation 30 min after the injection of the tracer. A large blood volume was withdrawn at the end to measure the whole-blood isotope concentration; 30 min was chosen to minimize the effect of intravascular tracer on brain ^{14}C activity measured at the end of the experiment, and, also, the eventual brain-to-blood reflux of tracer. The brain was rapidly removed and dissected into 8

specific regions according to Paxinos & Watson (1982). Whole blood, arterial plasma and weighed specimens of brain tissue were prepared for counting of radioactivity as previously described (Saija et al 1989, 1990a, b).

Regional blood volumes. We have calculated regional blood volumes to introduce a correction for the residual intravascular tracer. For each experimental group, four animals were used to evaluate regional correction for the blood volume in the dissected brain samples. Blood volume was defined as the [^{14}C]sucrose space at 2 min after the intravenous injection of the tracer (Gross et al 1982; Picozzi et al 1985; Saija et al 1990b). A 10 μCi bolus of [^{14}C]sucrose (5–15 mCi mmol^{-1}, Amersham) was injected intravenously. Two min later a blood sample was collected and the animal killed. Brain and whole-blood samples were digested and counted in the same way as [^{14}C]AIB.

Theory and calculation. A unidirectional blood-to-brain transfer constant (K_i) for AIB (Blasberg et al 1983a) was calculated, for each brain region examined, by the following equation developed by Ohno et al (1978) and Rapoport et al (1980):

$$K_i = \frac{C_i(T) - V\,C_b(T)}{\int_0^T C_p(t)dt} \quad (1)$$

where $C_i(T)$ is the tissue concentration of the tracer at the end of the experiment (nCi g^{-1}), T is the duration of the experiment (min), C_p is the arterial plasma concentration of the tracer (nCi mL^{-1}), V is the residual regional blood volume (μL g^{-1}) and $C_b(T)$ is the whole-blood tracer concentration (nCi mL^{-1}). Because K_i is related to PS (the permeability (P) – surface area (S) product; a parameter generally used to express cerebrovascular permeability because of difficulty in measuring S and blood flow (F) by $PS = F\ln(1 - K_i/F)$, and $PS << F$ for [^{14}C]AIB, K_i is independent of F and can be expressed in terms of plasma clearance (mL g^{-1} min^{-1} $\times 10^{-3}$).

Sucrose, a compound of very low permeability at the BBB, was employed as an intravascular marker to serve as an indicator for possible changes in the regional cerebral space after pharmacological challenges. The regional blood volume, V, was calculated as the ratio between the tracer concentration in the brain and in the whole blood according to the following equation:

$$V = \frac{C_i\ (\text{sucrose})}{C_b\ (\text{sucrose})} \quad (2)$$

where V is the cerebral blood volume (μL g^{-1}), C_i (sucrose) is the sucrose tracer concentration (nCi g^{-1}) in the brain and C_b (sucrose) is its concentration (nCi mL^{-1}) in the whole blood.

Pharmacological treatments. Rats were randomized and assigned to one of the following groups: Groups 1 and 2: 10–12 week old rats were injected intraperitoneally (i.p.) with haloperidol (Serenase, Lusofarmaco, Italy; 1 mg kg^{-1}) or its vehicle (distilled water; 1 mL kg^{-1}), respectively, 45 min before [^{14}C]AIB injection; Groups 3 and 4: 28–30-week old rats were given i.p. haloperidol (1 mg kg^{-1}) or its vehicle (distilled water; 1 mL kg^{-1}), respectively, 45 min before [^{14}C]AIB injection. Each group comprised 10 animals (6 rats given [^{14}C]AIB and 4 rats injected with [^{14}C]sucrose).

Statistical analysis. Results are expressed as mean ± s.e. of 4–6 determinations and compared using the nonparametric Mann-Whitney test.

Results

The K_i values for [^{14}C]AIB calculated in 8 brain regions of control animals (given haloperidol vehicle) were consistent with those described by other authors (Gross et al 1982; Picozzi et al 1985) and previously obtained in our laboratory (Saija et al 1989, 1990a, b).

The cerebral volume values used as a correction for the tracer still within the vessels at the end of the experiment did not differ significantly between all experimental groups (data not shown). Before isotope injection and throughout the experiment, arterial blood gases and pH were within normal limits in all animal groups (data not shown).

As shown in Table 1, the injection of haloperidol did not induce changes in the permeability of the BBB in 10–12-week old rats, but elicited a statistically significant increase of K_i values calculated in 28–30 week old animals (at the level of the occipital cortex, striatum, hippocampus and hypothalamus) and compared with those obtained in control animals (28–30-week old rats injected with the vehicle alone).

Comparison of regional K_i values in 28–30- and 10–12-week old control rats (given only haloperidol vehicle) showed a significant increase in the frontal and temporoparietal cortex, hypothalamus and cerebellum of the former group.

Discussion

The results of our study showed an enhanced vulnerability of the aged rats to a haloperidol-induced opening of the BBB. This is similar to the observation of Sankar et al (1983), who demonstrated an amphetamine-induced increase in the permeability of the BBB, which was greater in aged rats than in young animals.

Table 1. Transfer constants (K_i) for [^{14}C]AIB in male rats injected intraperitoneally with haloperidol (1 mg kg^{-1}) or its vehicle (distilled water).

	K_i (mL g^{-1} min^{-1} $\times 10^{-3}$)			
Brain area	10–12 weeks + vehicle	28–30 weeks + vehicle	10–12 weeks + haloperidol	28–30 weeks + haloperidol
Cortex				
frontal	1·65 ± 0·08	2·41 ± 0·05*	1·84 ± 0·11	2·64 ± 0·25‡
temporoparietal	1·68 ± 0·10	2·32 ± 0·07*	1·52 ± 0·11	2·31 ± 0·17‡
occipital	2·11 ± 0·06	2·17 ± 0·07	1·95 ± 0·14	2·66 ± 0·11†‡
Striatum	1·06 ± 0·04	1·09 ± 0·04	0·95 ± 0·04	1·50 ± 0·14†‡
Hippocampus	1·19 ± 0·08	1·29 ± 0·04	1·06 ± 0·05	1·96 ± 0·21†‡
Hypothalamus	3·18 ± 0·08	4·15 ± 0·12*	3·22 ± 0·21	4·80 ± 0·08†‡
Cerebellum	1·65 ± 0·11	2·19 ± 0·06*	1·73 ± 0·04	2·84 ± 0·37‡
Brain stem	1·71 ± 0·10	1·81 ± 0·06	1·65 ± 0·14	2·14 ± 0·18

*$P < 0.05$ 28–30 weeks + vehicle vs 10–12 weeks + vehicle.
†$P < 0.05$ 28–30 weeks + haloperidol vs 28–30 weeks + vehicle.
‡$P < 0.05$ 28–30 weeks + haloperidol vs 10–12 weeks + haloperidol.

Since the non-specific permeability of the BBB appeared locally increased in the older unchallenged rats in comparison with younger animals, one could explain the difference in the effects of haloperidol on the permeability of the BBB between these two groups of animals as due to a larger drug delivery to the brain in the older rats. We can rule out such hypotheses because the haloperidol-induced changes in the transport across the BBB in the aged rats do not overlap the pattern of differences in the BBB permeability observed between the unchallenged 10–12- and 28–30-week old rats. Pharmacokinetic changes might also contribute to the age-related effect of haloperidol on the permeability of the BBB; however, no difference in biodisposition of haloperidol was demonstrated in various strains of rats 3–4- to 11–12-months old (Campbell et al 1980; Wurzburger et al 1981; Kapetanovic et al 1982).

When screened in the traction test, in comparison with younger animals (data not shown), our older rats show a more marked sensitivity to the cataleptic effects of haloperidol, as also reported by Campbell & Baldessarini (1981). Since pharmacokinetic factors may be excluded, as reported before (Kapetanovic et al 1982), it would be interesting to ask if there is a relationship between the abnormal response of the BBB to the challenge with haloperidol and the increased sensitivity with age to the drug.

Several mechanisms are known to alter characteristics of the BBB; particularly, prolonged anoxia can damage endothelial cells of the BBB (Petito et al 1982). However, in our study, throughout the experiment, rats continued to have good colour and robust respiration, so that they did not suffer profound anoxia; in addition, no significant difference in blood pO_2, pCO_2 and pH was observed amongst experimental groups. Also, the existence of a relationship between the observed changes in BBB permeability and possible increments in cerebral blood flow may be excluded, because, in this mathematic model, the K_i for [^{14}C]AIB is independent of blood flow values (see Materials and methods); in addition, haloperidol, at a dose of 1 mg kg^{-1} i.p., produces a marked reduction in cerebral metabolism and blood flow, that is directly coupled to metabolic demand (Pizzolato et al 1985).

Disruption of normal function of the BBB to circulating solutes is usually the result of widened interendothelial junctions or alterations in one of the enzyme-like transport systems localized within the BBB. Nevertheless, it has been shown that amino acids which have a high brain uptake index have a greater affinity for their BBB carrier system than those exhibiting a low index, such as AIB (Oldendorf 1971). Thus, because [^{14}C]AIB transport across the BBB is not facilitated, it is unlikely that changes in K_i values are due to a tracer sequestration into endothelial cells and not to widened interendothelial junctions (Saija et al 1990a).

References

Blasberg, R. G., Gazendam, J., Patlak, C. S., Fenstermaker, J. D. (1980) Quantitative autoradiographic studies of brain edema and a comparison of multi-isotope autoradiographic techniques. In: Cervos-Navarro, J., Ferszt, R. (eds) Advances in Neurology, Vol. 28: Brain Edema. Raven Press, New York, pp 255–270

Blasberg, R. G., Fenstermacher, J. D., Patlak, C. S. (1983a) Transport of α-aminoisobutyric acid across brain capillary and cellular membranes. J. Cereb. Blood Flow Metab. 3: 8–32

Blasberg, R. G., Patlak, C. S., Fenstermacher, J. D. (1983b) Selection of experimental conditions for the accurate determination of blood-brain transfer constants from single-time experiments: a theoretical analysis. Ibid. 3: 215–225

Campbell, A., Baldessarini, R. J. (1981) Effects of maturation and aging on behavioural responses to haloperidol in the rat. Psychopharmacology 73: 219–222

Campbell, A., Herschel, M., Cohen, B. M., Baldessarini, R. J. (1980) Tissue levels of haloperidol by radioreceptor assay and behavioral effects of haloperidol in the rat. Life Sci. 27: 633–640

Ellison, M. D., Povlishock, J. T., Hayes, R. L. (1986) Examination of the blood-brain barrier transfer of α-aminoisobutyric acid and horseradish peroxidase: regional alterations in blood-brain barrier function following acute hypertension. J. Cereb. Blood Flow Metab. 6: 471–480

Gross, P. M., Teasdale, G. M., Graham, D. I., Angerson, W. J., Harper, A. M. (1982) Intra-arterial histamine increases blood-brain transport in rats. Am. J. Physiol. 243: H307–H317

Kapetanovic, I. M., Sweeney, D. J., Rapoport, S. I. (1982) Age effects on haloperidol pharmacokinetics in male, Fisher-344 rats. J. Pharmacol. Exp. Ther. 221: 434–438

Ohno, K., Pettigrew, K., Rapoport, S. (1978) Lower limits of cerebrovascular permeability to nonelectrolytes in the conscious rat. Am. J. Physiol. 235: 299–307

Oldendorf, W. H. (1971) Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. Ibid. 221: 1629–1638

Pardridge, W. M. (1987) The gate keeper: how molecules are screened for admission to the brain. The Sciences 27: 50–55

Pardridge, W. M. (1988) Recent advances in blood-brain barrier transport. Ann. Rev. Pharmacol. Toxicol. 28: 25–39

Paxinos, G., Watson, C. (1982) The rat brain in stereotaxic coordinates. Academic Press, New York

Petito, C. K., Pulsinelli, W. A., Jacobson, G., Plum, F. (1982) Edema and vascular permeability in cerebral ischemia: comparison between ischemic neuronal damage and infarction. J. Neuropathol. Exp. Neurol. 41: 423–426

Picozzi, P., Todd, N., Crockard, A. (1985) Regional blood-brain barrier permeability changes after restoration of blood flow in postischemic gerbil brains: a quantitative study. J. Cereb. Blood Flow Metab. 5: 10–16

Pizzolato, G., Soncrant, T. T., Holloway, H. W., Rapoport, S. I. (1985) Reduced metabolic response of the aged rat brain to haloperidol. J. Neurosci. 5: 2831–2838

Rapoport, S. I. (1976) Blood-brain barrier in physiology and medicine. Raven Press, New York

Rapoport, S. I., Hori, M., Klatzo, I. (1972) Testing of a hypothesis for osmotic opening of the blood-brain barrier. Am. J. Physiol. 223: 323–331

Rapoport, S. I., Fredericks, W. R., Ohno, K., Pettigrew, K. D. (1980) Quantitative aspects of reversible osmotic opening of the blood-brain barrier. Ibid. 238 (Regulatory Integrative Comp. Physiol. 7): R421–R431

Saija, A., Princi, P., De Pasquale, R., Costa, G. (1989) Modifications of the permeability of the blood-brain barrier and local cerebral metabolism in pentobarbital- and ketamine-anaesthetized rats. Neuropharmacology 28: 997–1002

Saija, A., Princi, P., D'Amico, N., De Pasquale, R., Costa, G. (1990a) Aging and sex influence the permeability of the blood-brain barrier in the rat. Life Sci. 47: 2261–2267

Saija, A., Princi, P., De Pasquale, R., Costa, G. (1990b) Arecoline, but not haloperidol, induces changes in the permeability of the blood-brain barrier in the rat. J. Pharm. Pharmacol. 42: 135–138

Sankar, R., Blossom, E., Clemons, K., Charles, P. (1983) Age-associated changes in the effects of amphetamine on the blood-brain barrier of rats. Neurobiol. Aging 4: 65–68

Wurzburger, R. J., Miller, R. L., Marcum, E. A., Colburn, W. A., Spector, S. (1981) A new radioimmunoassay for haloperidol: direct measurement of serum and striatal concentrations. J. Pharmacol. Exp. Ther. 217: 757–763