Table 3. Effect of different diets on in vitro absorption ^{45}Ca in rats

Diet	^{45}Ca absorbed (CPM)*	Relative percentage of absorption of ^{45}Ca
D_1-Wheat chapati	34.17 ± 6.15	32.4 (36.7)**
D_2-Wheat & Bengal gram (80:20) chapati	81.77 ± 10.58	77.5
D_3-Wheat & Bengal gram (70:30) chapati	92.99 ± 8.81	88.2
D_4-Casein	105.51 ± 7.94	100.0
C.D. at 5%	31.24	

* These are the values expressed as ^{45}Ca counts per min (CPM)/100 µl of serosal fluid/100 mg fresh tissue/30 min incubation at 37 °C.
** The value given in parentheses is the ^{45}Ca absorption as a percentage of the value for the D_3 diet fed group.

incubation at 37 °C. Results were statistically analysed for analysis of variance.

Results and discussion

The results indicate (table 3) that the rats fed on wheat and Bengal gram (80:20 and 70:30) chapati diets (D_2 and D_3) absorbed more than twice the amount of ^{45}Ca (CPM/100 µl of serosal fluid/100 mg tissue) as compared to the group fed on a wheat chapati diet (D_1). The difference in ^{45}Ca absorption between groups with wheat and Bengal gram diets (D_2 and D_3) and those with casein diet (D_4) was nonsignificant. The absorption of ^{45}Ca in the wheat diet group (D_1) was only 36.7% of that for the wheat and Bengal gram (70:30) diet (D_3). The data clearly reveal that the supplementation of cereal diets with Bengal gram significantly improved calcium absorption, and this may be due to the improved amino acid composition of these diets. It has been reported that the deficiency of essential amino acids in the diet may cause a drop in the calcium-binding activity of muco-proteins [13] and in calcium absorption in vitro and in vivo.

The protein content was higher and there was less phytin phosphorus in wheat and Bengal gram (80:20 and 70:30) chapatis as compared to wheat chapati (table 1). As all the diets fed to rats were prepared at 10% protein level, the total content of wheat in the wheat chapati diet was higher (see table 2). The diets which contained Bengal gram therefore had a lower phytic acid content. This may also have contributed to the better calcium absorption observed in vitro. As the consumption of animal proteins over long periods is known to produce some deleterious effects, cereal-legume mixed diets may be used in place of animal proteins without much effect on the calcium absorption.

* Dept of Vet. Physiology, Punjab Agricultural University, Ludhiana (India)

1 Robertson, W. G. Peacock, M., Heyburn, P. J., Speed, R., and Hanes, F., Fortschr. Urol. Nephrol. *11* (1978) 5.
2 Robertson, W. G., and Peacock, M., Urol. Int. *37* (1982) 394.
3 Lewis, A. F., Br. med. J. *283* (1981) 1217.
4 A.O.A.C., Official Methods of Analysis. Association of Official Analytical Chemists. 12th Edn. Washington, D.C. (1980).
5 Goering, H. K., and Vansoest, P. J., Forage-fibre Analysis. Agricultural Handbook Series No. 379, USDA, Washington, D.C. (1970).
6 Tara, K. A., Usha, M. S., Prabhavati, C., and Bains, G. S., Res. Ind. *16* (1971) 96.
7 Willis, J. B., Analyt. Chem. *33* (1961) 556.
8 Akedo, H., Sugawa, T., Yoshi Kawa, S., and Suda, M. J., Biochemistry *47* (1960) 124.
9 Chapman, D. G., Castillo, R., and Campbell, J. A., J. Biochem. Physiol. *37* (1959) 679.
10 Bernhart, F. W., and Tomareli, R. M., J. Nutr. *89* (1966) 496.
11 Bray, G. A., Analyt. Biochem. *1* (1960) 279.
12 Kawatra, B. L., and Bhatia, I. S., Biochem. Physiol. Pflanz. *174* (1979) 283.
13 Tazhibaev, N. S., Frgaliena, A. A., and Mamyrbaev, A. A., Nutr. Abstr. Rev. *47* (1977) 775.

0014-4754/90/101016-02$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1990

Irradiation of the head by ^{60}Co opens the blood-brain barrier for drugs in rats

Š. Bezek [a], T. Trnovec [a,*], V. Ščasnár [a], M. Ďurišová [a], M. Kukan [a], Z. Kállay [a], V. Laginová [b] and V. Svoboda [c]

[a] *Institute of Experimental Pharmacology, Slovak Academy of Sciences, CS-84216 Bratislava,* [b] *Institute of Clinical Oncology, Bratislava, and* [c] *Purkynje Medical Academy, Hradec Králové (Czechoslovakia)*
Received 5 February 1990; accepted 1 June 1990

Summary. The passage of 6 model drugs; acetylsalicylic acid, chloramphenicol, ethimizol, carbisocaine, heptacaine, and diazepam, through the blood-brain barrier, was determined in unirradiated control rats and in animals 1, 3, and 7 days after irradiation of the head only with a dose of 25 Gy from a ^{60}Co source. The brain uptake index (BUI), which compares the uptake of the test substance with that of ^3H_2O 5 s after their injection into the common carotid artery, was significantly increased in comparison with unirradiated controls 7 days after irradiation, for all substances tested except for ethimizol. For acetylsalicylic acid and chloramphenicol it was also significantly increased in the other time intervals. The less lipophilic substances showed a greater relative increase of BUI than the more lipophilic ones.
Key words. Blood-brain barrier; irradiation; drugs.

Ionizing radiation affects the integrity of the blood-brain barrier (BBB). This has been shown after irradiation of the head by a broad spectrum of radiation doses, and the damage to the BBB could be observed immediately or within several months after irradiation. Various tracers, such as ^{131}I-human serum albumin, horse-radish peroxidase, trypan blue, Evans' blue, acid fuchsin, uranin, fluorescein, serotonin, ^{35}S-sulfate, ^{32}P-phosphate, and ^{14}C-urea, have been used to demonstrate the radiation-induced permeability changes in the BBB. The vast amount of data published on this topic has been surveyed by Antipov et al.[1]. On the other hand, data on changes in permeability of drugs through the BBB are scarce. Increased permeability for acetazolamide[2], methotrexate[3–5], Na penicillin[6], and galacticol[7] have been reported. The permeability of drugs through the intact BBB is related to their physico-chemical properties[8]. A correlation was found to exist between the brain uptake index (BUI) and the octanol-saline partition coefficient[9]. However, it is not known how radiation modifies this permeability pattern. In spite of electron microscopic correlates[10–15], the mechanisms underlying radiation-induced changes in BBB permeability for drugs are not clear.

The aim of this work was to demonstrate the time-course of permeability alterations induced by head-irradiation of rats for six model drugs with differing properties.

Material and methods

The BBB penetrability was studied by the method of Oldendorf[16]. Male Wistar rats (220–280 g) were anesthetized with pentobarbital. The common carotid artery was surgically exposed and cannulated with a needle (o.d. 0.4 mm). An intra-arterial bolus injection (0.2 ml vol.) was administered, containing tritiated water as a diffusible reference (100–150 kBq), the ^{14}C-test substance (15–20 µg and 30–50 kBq) and a plasma volume reference ^{113}In-EDTA (100–150 kBq) in phosphate-buffered saline. The rats were decapitated 5 s after injection. The brain was removed and the hemisphere ipsilateral to the injection was prepared for scintillation counting.

The radioactivity of ^{113m}In, ^{14}C and ^{3}H was determined using a Packard TriCarb 300 CD liquid scintillation counter[17]. The rats were irradiated from a ^{60}Co source with a dose of 25 Gy. The source-to-skin distance was 60 cm and the dose rate was 1.88 Gy/min. During irradiation the animals were restrained in lead holders, and shielded by 55 mm Pb except for the head. BUI was determined in control rats and in treated animals (1, 3 or 7 days after irradiation) according to the equation[16]:

$$\mathrm{BUI}/(100\%) = \frac{\dfrac{^{14}\text{C test substance}}{^{3}\text{H water}}\,(\text{brain})}{\dfrac{^{14}\text{C test substance}}{^{3}\text{H water}}\,(\text{mixture})} - \frac{\dfrac{^{113m}\text{In-EDTA}}{^{3}\text{H water}}\,(\text{brain})}{\dfrac{^{113m}\text{In-EDTA}}{^{3}\text{H water}}\,(\text{mixture})}$$

The partition coefficients octanol: phosphate-buffered saline pH 7.4 of the test substances (for their molecular weights see table 1) were determined[9].

Radiochemicals: Heptacaine, a substance with local anesthetic[18,19] and antiarrhythmic[20] properties, weak base, $pK_a = 8.9$; carbisocaine, a substance with local anesthetic activity[21,22], weak base, $pK_a = 8.9$; ethimizol[23,24], a respiratory analeptic and nootropic drug of Soviet make, weak base, $pK_a = 1.75$[25]. (Heptyl-1-^{14}C)-heptacaine, (heptyl-1-^{14}C)-carbisocaine[26], tritiated water and ^{113m}In generator were supplied by the Institute for Research, Production and Use of Radioisotopes, Prague, Czechoslovakia. Each 1.0 ml of ^{113m}In eluted from the generator was chelated by 10 µl of Na_2EDTA solution (15% w/v). Acetyl (carboxyl-^{14}C) salicylic acid, $pK_a = 3.5$[27]; (2-^{14}C)diazepam, a weak base, $pK_a = 3.3$[27]; D-threo-(dichloroacetyl-1-^{14}C) chloramphenicol, an alcohol (diol), $pK_a = 5.5$[27], were supplied by Amersham International plc, Amersham, England. 2-^{14}C-Ethimizol[28] was supplied by the Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad, USSR.

Arithmetic means and their errors were calculated. Student's t-test was used for testing differences between the groups.

Table 1. The brain uptake index for six model test substances in unirradiated rats

Substance	MW	Octanol: saline partition coefficient	n	BUI ± SEM in unirradiated control rats
Acetylsalicylic acid	300.3	0.09	5	4.1 ± 1.1
Ethimizol	210.0	3.75	7	101.2 ± 0.7
Chloramphenicol	323.1	13.98	8	9.7 ± 0.7
Carbisocaine	401.0	71.18	11	57.7 ± 3.9
Heptacaine	399.0	155.75	6	62.2 ± 3.6
Diazepam	284.8	312.28	5	94.4 ± 3.7

Results and discussion

Table 1 shows molecular weights and the octanol-saline partition coefficients of the test substances as well as the BUIs for unirradiated rats. It can be seen that BUI normalized to the square root of molecular weight increases proportionally with the partition coefficient, except for ethimizol. Linear regression analysis indicated that the relationship[9] could be defined thus:

$$(\log \mathrm{BUI})\sqrt{\text{molecular weight}} = 7.3\ (\log \mathrm{K}) + 17.7,$$

where K is the partition coefficient. Standard errors (SD) of the values for the slope and intercept were 2.1 and 3.5, respectively. Of the drugs studied in our series, only

Table 2. The brain uptake index for six model test substances 1, 3, and 7 days after isolated head irradiation with a dose of 25 Gy from a ^{60}Co source in rats

Substance	Day 1 BUI ± SEM	% control	n	Day 3 BUI ± SEM	% control	n	Day 7 BUI ± SEM	% control	n
Acetylsalicylic acid	10.7 ± 0.7*	259.9	6	11.5 ± 2.1*	278.5	3	14.9 ± 3.8*	360.9	6
Ethimizol	101.2 ± 3.7	100.0	6	108.8 ± 6.8	107.5	6	105.1 ± 2.4	103.9	9
Chloramphenicol	13.5 ± 1.1*	139.5	5	15.0 ± 3.0	155.1	7	25.2 ± 6.4*	259.9	5
Carbisocaine	55.4 ± 4.9	95.9	4	72.1 ± 11.9	124.9	4	81.0 ± 6.6*	140.3	4
Heptacaine	64.8 ± 11.3	104.0	4	75.9 ± 10.8	122.0	4	100.2 ± 9.1*	161.1	5
Diazepam	94.8 ± 7.0	100.4	4	102.6 ± 4.9	108.1	4	136.5 ± 8.9*	144.6	5

* Significantly different from controls ($p < 0.05$).

acetylsalicylic acid was among the 47 substances investigated by Cornford et al.[9], who obtained the following relationship:

$$(\log \text{BUI}) \sqrt{\text{molecular weight}} = 6.0 (\log K) + 14.5.$$

The SD of parameter estimates was 0.5 and 0.8, respectively. An equal correlation of 0.86 was found between the lipophilic properties and uptake of the substances in the brain for both data sets. It can be seen from table 2 that irradiation of the head was followed by an increase of BUI of all test substances with the exception of ethimizol. The percentage increase of BUI was greater with less lipophilic substances than with more lipophilic ones. The brain uptake of highly permeating drugs is limited by blood flow, whereas the uptake of drugs of low permeability is limited mainly by the permeability of the barrier[29]. Thus the changes of BUI for moderately lipophilic substances could be due either to permeability modification or to blood flow changes. In another experiment (prepared for publication), we observed no changes in regional cerebral blood flow in rats after isolated irradiation of the head with a dose as great as 180 Gy. It may thus be assumed that with moderately lipophilic test compounds, alteration of the permeability of the brain capillaries and not of blood flow was the factor responsible for the increase of BUI after irradiation.

Among the substances studied, the BUI of ethimizol seems not to be related to the value of its partition coefficient. It was previously shown that shortly after administration ethimizol uniformly distributed in organs and tissues including brain[30]. The transfer of ethimizol into hepatocytes was instantaneous, and was independent of both temperature and metabolic inhibitors[31]. These findings suggest that ethimizol might pass across biological membranes by simple diffusion. However, in solution in water ethimizol forms a very stable conformation, a fragment of which is identical with adenine[32]. A carrier-mediated BBB transport of adenine has been described[33]. It may be hypothesized that ethimizol is transported into the brain by the adenine carrier. Moreover, caffeine, to which ethimizol is structurally related, enters the brain both by simple diffusion and by saturable, carrier-mediated transport and was found to cause a dose-dependent inhibition of ^{14}C-adenine transport[34].

The greatest changes in penetration of the test substances across the BBB were found 7 days after irradiation. Compared to radiation-induced permeability changes reported[1-6], the changes in our experiment were of longer duration, conceivably because of the higher radiation dose administered. The time-course in our data is more similar to that obtained after focal doses of 60 Gy[35].

The relationship between BUI and lipophilic properties, shown to be valid for unirradiated rats, was calculated also for days 1, 3, and 7 after irradiation. Compared to the intercept of 17.7 ± 3.3 for the control rats, intercepts of 20.8 ± 2.9, 21.5 ± 3.0, and 23.0 ± 2.3 were found for the days, 1, 3 and 7 after irradiation, respectively. The increase of intercept is related to the more pronounced increase of BUI for less lipophilic substances after irradiation. In agreement with this, the slopes for irradiated rats were lower than those for controls (cf. 7.3 ± 1.9 for controls with 5.9 ± 1.7, 6.3 ± 1.8, and 6.6 ± 1.4 for the 1st, 3rd, and 7th day). It may be concluded that a single irradiation of the head of rats with a radiologically relevant dose of 25 Gy opened the blood-brain barrier for a group of 6 test substances. Relatively greater changes were recorded with less lipophilic tracers.

* To whom correspondence should be addressed.

1 Antipov, V. V., Davydov, B. I., and Ushakov, I. B., Kosm. Issled. *22* (1984) 297.
2 Nair, V., and Roth, L. J., Radiat. Res. *23* (1964) 265.
3 Griffin, T. W., Rasey, J. S., and Bleyer, W. A., Cancer *40* (1977) 1109.
4 Mahler, P. A., Abstracts 32. A. Sci. Meeting Radiat. Society, Orlando, Florida 1984, p. 113. Radiation Research Soc., Philadelphia 1984.
5 Storm, A. J., Van Der Kogel, A. J., and Nooter, K., Eur. J. Cancer clin. Oncol. *21* (1985) 759.
6 Remler, M. P., and Marcussen, W. H., Exp. Neurol. *73* (1981) 310.
7 Levin, V. A., Edwards, M. S., and Byrd, A., Int. J. Radiat. Oncol. Biol. Phys. *5* (1979) 1627.
8 Oldendorf, W. H., Proc. Soc. exp. Biol. Med. *147* (1974) 813.
9 Cornford, E. M., Braun, L. D., Oldendorf, W. H., and Hill, M. A., Am. J. Physiol. *243* (1982) 161.
10 Ostenda, M., Acta physiol. pol. *30* (1979) 455.
11 Abdrakhmanov, A. A., Kachurin, A. L., Mashanskiy, V. F., Postnikov, L. N., and Sverdlov, A. G., Byul. Eksp. Biol. Med. *101* (1986) 622.
12 Abdrakhmanov, A. A., and Otellin, V. A., Arkh. Anat. Gistol. Embriol. *92*(2) (1987) 17.
13 Kimura, Y., Ogawa, Y., Mitsuhashi, K., Honda, Y., Kinki Daigaku Rikogakubu Kenkyu Hokoku no. 22 (1986) 247.
14 Kamarád, V., Acta univ. palackianae olomucensis *114* (1986) 123.
15 Kamarád, V., Svoboda, V., and Dosoudilová, M., Acta univ. palackianae olomucensis *117* (1987) 65.
16 Oldendorf, W. H., A. Rev. Pharmac. *14* (1974) 239.
17 Oldendorf, W. H., and Szabo, J., Am. J. Physiol. *230* (1976) 94.
18 Čižmárik, J., Borovanský, A., and Švec, P., Pharmazie *33* (1978) 297.
19 Pešák, M., Kopecký, F., Čižmárik, J., and Borovanský, A., Pharmazie *35* (1980) 150.

20 Kozlovský, J., Čižmárik, J., Pešák, M., Inczinger, F., and Borovanský, A., Arzneim.-Forsch. *32* (1982) 1032.
21 Beneš, L., Borovanský, A., and Kopáčová, L., Archs Pharm. *9* (1972) 648.
22 Beneš, L., Borovanský, A., and Kopáčová, L., Arzneim.-Forsch./Drug Res. *19* (1969) 1902.
23 Vinogradova, N. B., Khromov-Borisov, N. V., Kozhevnikov, S. P., and Livshits, I. M., Zh. Obshch. Kim. *31* (1961) 1471.
24 Bordokin, Yu. S., and Zaitsev, Yu. V., Sechenov Fiziol. Zh. SSSR *70* (1984) 983.
25 Piotrovskiy, L. B., Ivanova, I. A., and Chernik, G. G., Khim. Farm. Zh. *43* (1984) 129.
26 Elbert, T., Marko, V., Filip, J., and Beneš, L., J. Label. comp. Radiopharm. *21* (1984) 101.
27 Heel, R. C., and Avery, G. S., in: Drug Treatment, p. 1211, Ed G. S. Avery. Adis Press, Sydney 1980.
28 Usaevich, Yu. Ya., and Vekshina, L. I., Khim. Farm. Zh. *11/10* (1977) 37.
29 Bradbury, M. W. B., Patlak, C. S., and Oldendorf, W. H., Am. J. Physiol. *229* (1975) 1110.
30 Trnovec, T., Ďurišová, M., Bezek, Š., Burdátš, P., Marko, V., Faberová, V., Zemánek, M., Šoltés, L., and Piotrovskiy, L. B., Drug Metab. Dispos. *14* (1986) 718.
31 Bezek, Š., Kukan, M., Kállay, Z., Trnovec, T., Štefek, M., and Piotrovskiy, L. B., Drug Metab. Dispos. *18* (1990) 88.
32 Khromov-Borisov, N. V., Borisova, G. Yu., Aleksandrova, I. Ya., Goldfarb, V. L., Brovcina, N. V., Zaitsev, Yu. V., and Borodkin, Yu. S., Zh. Vyssh. Nerv. Deyat. I.P. Pavlova *28* (1978) 761.
33 Cornford, E. M., and Oldendorf, W. H., Biochim. biophys. Acta *394* (1975) 211.
34 McCall, A. L., Millington, W. R., and Wurtman, R. J., Life Sci. *31* (1982) 2709.
35 Remler, M. P., and Marcussen, W. H., Appl. Neurophysiol. *46* (1983) 276.

0014-4754/90/101017-04$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1990

Presence of 3,4-dihydroxyphenylalanine-containing peptides in hemocytes of the ascidian, *Halocynthia roretzi*

K. Azumi, H. Yokosawa * and S. Ishii

Department of Biochemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060 (Japan)
Received 21 February 1990; accepted 3 May 1990

Summary. Five 3,4-dihydroxyphenylalanine (DOPA)-containing peptides have been isolated from hemocytes of the ascidian, *Halocynthia roretzi*. Three of them were composed of DOPA, proline, phenylalanine, histidine and arginine in different ratios, while the other two contained only DOPA and an unidentified amino acid. DOPA-containing peptides were found to exist in only one type of hemocyte.
Key words. 3,4-dihydroxyphenylalanine; DOPA-containing peptide; hemocyte; ascidian; boronate immobilized column.

Two 3,4-dihydroxyphenylalanine (DOPA)-containing proteins have been isolated from tissues and hemocytes of invertebrates [1-4]. One protein with a molecular weight of 130,000, isolated from the phenol gland of the marine mussel, *Mytilus edulis*, contains 11% DOPA and a large amount of hydroxyproline (13%)[1,2]. The other is named ferreascidin; this has been isolated from hemocytes of the stolidobranch ascidian, *Pyura stolonifera*, and has 17% DOPA and a large amount of tyrosine (42%)[3,4]. The molecular weight is about 10,000. Furthermore, modified tripeptides containing DOPA and/or hydroxy-DOPA and compounds related to them have been isolated from hemocytes of the phlebobranch ascidian, *Ascidia nigra*, and the stolidobranch ascidian, *Molgula manhattensis*, as reducing blood pigments[5,6]. They are designated as tunichromes.

In a previous communication[7], we have reported the presence of two DOPA-containing tetrapeptide-like substances named halocyamine A and B in the acetone-extract of hemocytes of the stolidobranch ascidian, *Halocynthia roretzi*. Halocyamine A consists of DOPA, histidine, glycine, and a tryptophan derivative, while B consists of DOPA, histidine, threonine, and the same tryptophan derivative. They are present in only one type of hemocyte and show antibacterial activity. Thus, we have proposed that they may play important roles in the defense mechanisms of *H. roretzi*.

The demonstrations[3,4] showing the presence of DOPA-containing proteins in hemocytes of the ascidian of the stolidobranch led us to hypothesize that halocyamines would be derived from putative DOPA-containing precursors present in *H. roretzi* hemocytes because *H. roretzi* belongs to the same suborder, the stolidobranch. In the course of studies undertaken to test the hypothesis, we found DOPA-containing peptides other than halocyamines in the aqueous extract of the hemocytes. In this paper, we describe the isolation and characterization of DOPA-containing peptides of *H. roretzi* hemocytes.

Materials and methods

Solitary ascidians *H. roretzi*, type C, were harvested in Mutsu Bay, Japan. Hemocytes were collected as described previously[7] and were frozen at − 20 °C until used.