with the SCG; either as a part of the sympathetic preganglionic trunk, or as cholinergic fibers originating near, or in synaptic proximity with the preganglionic sympathetics. On the basis of histochemical studies, others have denied such a possibility, since the large stores of choline acetyltransferase, which appear to be associated with vessels that relax upon transmural stimulation in vitro, are not depleted by section of the sympathetic nerves supplying these regions[2, 10]. Should this be the case, it is conceivable that the trigger for the release of vasodilating transmitter is hemodynamic rather than neurogenic in nature. A local reflex for maintaining central perfusion pressure and regional blood flows during stress has been proposed by others[12] and the present study may provide support for this concept.

1 Tuttle, R.S., Am. J. Physiol. *248* (1985) H867.
2 Bevan, J.G., Buga, G., Jope, C.A., Jope, R.S., and Moritoki, H., Circulation Res. *51* (1982) 421.
3 Brayden, J.E., and Bevan, J.A., Circulation Res. *56* (1985) 205.
4 Lee, T.T.-F., Hume, W.R., Sue, C., and Bevan, J.A., Circulation Res. *42* (1978) 535.
5 Beck, L., and Brody, M.J., Angiology *12* (1961) 202.
6 Beck, L., Pollard, A.A., Kayaalp, A.O., and Weiner, L.W., Fedn Proc. *25* (1966) 1596.
7 Pollard, A.A., and Beck, L., J. Pharm. expl Ther. *179* (1971) 132.
8 Rosen, A., Acta physiol. scand. *52* (1961) 291.
9 Bevan, J.A., Buga, G.M., Florence, V.M., Gonsalves, A., and Snowden, A., Circulation Res. *50* (1982) 470.
10 Lee, T.T.-F., Circulation Res. *9* (1971) 971.
11 Uvnas, B., Physiol. Rev. *40* (1960) 69.
12 Bevan, J.A., Bevan, R.D., Buga, G., Florence, V.M., Jope, C.A., Jope, R.S., and Moritoki, H., in: Vasodilation: The Cranial Neural Vasodilator Outflow, p. 19. Eds P.M. Vanhoute and I. Leusen. Raven Press, New York 1981.

0014-4754/86/070817-03$1.50 + 0.20/0

Hydroxyproline concentration in soluble and insoluble material from serum treated with trichloroacetic acid in postpartum mice

K. Shimizu, K. Honda, S. Takabe and M. Hokano

Department of Anatomy, Tokyo Medical College, Shinjuku, Shinjuku-ku, Tokyo 160 (Japan), 27 February 1984

Summary. The hydroxyproline concentration in both the soluble and insoluble material from trichloroacetic acid-treated serum from postpartum mice was determined. The hydroxyproline concentration in the insoluble material increased, but that in the soluble material did not increase during the uterine involuting period.
Key words. Hydroxyproline concentration; serum; trichloroacetic acid; postpartum; mice.

In the mouse, most of the uterine collagen is degraded during the first two postpartum days[1-3]. It is generally thought that collagenase initially degrades collagen fibers to fragments and then other proteolytic enzymes break down these fragments to amino acids[4, 5]. It is not clear to what extent these proteolytic enzymes participate in uterine collagen degradation during the postpartum involution.

Since materials derived from the breakdown of the uterine collagen are removed by the blood stream[6, 7], the occurrence of material containing hydroxyproline (Hyp) in the serum should reflect the collagen-degrading process in the postpartum uterus. We undertook to determine the Hyp concentration in both soluble and insoluble material from serum treated with trichloroacetic acid (TCA), which can fully precipitate large peptides[8], during the first three postpartum days.

Materials and methods. Animals used were female mice of the IVCS strain. They were reared under 12L:12D and given food and water ad libitum. At 8 weeks of age they were mated. All animals were allowed to suckle their pups. The day of parturition was indicated as day 0 postpartum.

Our previous studies[1-3] showed that most of the uterine collagen is degraded during the first two postpartum days. Therefore, blood was collected from day 0 to day 3 postpartum from the femoral artery and vein under ether anesthesia. Only blood samples taken from mice having 8–12 placental scars per pair of uterine horns were analyzed, to avoid any difference due to the number of placental scars[6]; the average number of placental scars in a pair of horns was 9.3 (n = 120). Blood samples taken from 10-week-old diestrous virgin mice served as controls. All blood samples were centrifuged at 3000 rpm for 20 min. The sera of 5 mice were pooled and stored at −20 °C.

One ml 10% (W/V) TCA was added to 1 ml of the serum, which was then centrifuged at 3000 rpm for 20 min. One ml 12 N HCl was added to 1 ml supernatant. The sediment was dried at 100 °C for 24 h and weighed and 2 ml 6 N HCl was added. The samples were hydrolyzed at 130 °C for 3 h. Hyp was determined in these hydrolysates according to Woessner's method[9].

Hydroxyproline concentration in soluble and insoluble material from serum treated with 10% (W/V) trichloroacetic acid during the first three postpartum days

Day after parturition	No of animals[a]	Hydroxyproline concentration Soluble material µg/ml	Insoluble material (dry weight) mg	µg/g
Nulliparous	30	5.1 ± 1.5[b]	55.3 ± 1.1	61.9 ± 8.0
Day 0	30	2.0 ± 0.2	58.9 ± 0.9*	39.4 ± 7.5
Day 1	30	3.0 ± 0.6	66.1 ± 2.6**	83.2 ± 3.8*
Day 2	30	2.1 ± 0.5	71.0 ± 2.2**	92.0 ± 10.3*
Day 3	30	1.5 ± 0.5*	55.3 ± 1.2	50.9 ± 8.5

[a] The serum from 5 mice was pooled. [b] mean ± SE. *$p < 0.05$, **$p < 0.01$ as compared with nulliparous animals (Student's t-test).

Results and discussion. Results are summarized in table 1. The Hyp concentration of the supernatant was the same as that of nulliparous animals from day 0 to day 2 postpartum. The Hyp concentration was decreased on day 3 postpartum. A small increase of the Hyp concentration was found on day 1 postpartum during the first three postpartum days.

The dry weight of the sediment was higher than that of sediment from nulliparous animals from day 0 to day 2 postpartum. The dry weight decreased to the level of that of nulliparous animals on day 3 postpartum. The Hyp concentration of the sediment was not increased on day 0 postpartum but was high on day 1 and day 2 postpartum compared with that of nulliparous animals. The Hyp concentration decreased to the level of that in nulliparous animals on day 3 postpartum.

Our results indicated that the greater part of the Hyp in the serum is not present as free amino acid, and that most of the proteolytic enzymes which can degrade large peptides to amino acids[4,5] do not participate in the postpartum collagen degradation. The increase of both the Hyp concentration and the dry weight of the sediment is consistent with our previous reports[1-3] that most of the uterine collagen degrades during the first two postpartum days.

These results are in accordance with previous observations that the uterine collagenase activity is high during the first two postpartum days[10,11], that the purified uterine collagenase can degrade collagen fibers to small peptides[12], and that a lysosomal proteolytic enzymic activity (cathepsin) is low during the period of rapid degradation of collagen[13].

In summary, uterine collagenase degrades collagen fibers to small peptides and then small peptides are removed by the blood stream.

1 Shimizu, K., Furuya, T., Takeo, Y., Shirama, K., and Maekawa, K., Acta anat. *116* (1983) 10.
2 Shimizu, K., Furuya, T., Takeo, Y., Shirama, K., and Maekawa, K., Zool. Mag., Tokyo *92* (1983) 219.
3 Shimizu, K., and Maekawa, K., Acta anat. *117* (1983) 257.
4 Pérez-Tamayo, R., Am. J. Path. *92* (1978) 508.
5 Sellers, A., and Murphy, G., Int. connect. Tissue Res. *9* (1981) 151.
6 Harkness, R. D., and Moralee, B. E., J. Physiol., London *132* (1956) 502.
7 Woessner, J. F. Jr, Biochem. J. *83* (1962) 304.
8 Fruton, J. S., in: The Protein, vol. 1, p. 189. Ed. H. Neurath. Academic Press, New York 1963.
9 Woessner, J. F. Jr, Archs Biochem. Biophys. *93* (1961) 440.
10 Jeffrey, J. J., Coffey, R. J., and Eisen, A. Z., Biochim. biophys. Acta *252* (1971) 136.
11 Halme, J., and Woessner, J. F. Jr, J. Endocr. *66* (1975) 357.
12 Roswit, W. T., Halme, J., and Jeffrey, J. J., Archs Biochem. Biophys. *225* (1983) 285.
13 Woessner, J. F. Jr, Biochem. J. *97* (1965) 855.

0014-4754/86/070819-02$1.50 + 0.20/0

Regional pattern and heat-resistance of brain 5′-deoxy-5′-methylthioadenosine phosphorylase

A. Abbruzzese*, G. Della Pietra and R. Porta

Dept of Biochemistry, 1st Medical School, University of Naples, Naples (Italy), 30 September 1985

Summary. The distribution of 5′-deoxy-5′-methylthioadenosine (MTA) phosphorylase in 10 pig brain areas was determined. The observed regional differences of the enzymatic activity seem to reflect more the pattern of brain spermine distribution rather than that of spermidine. Moreover, comparative studies on the heat-resistance of MTA phosphorylase extracted from the whole brain of various species suggest structural differences in the enzyme molecules occurring in the brains of different animals.
Key words. Polyamines; methylthioadenosine; methylthioadenosinephosphorylase; brain; regional distribution.

5′Deoxy-5′-methylthioadenosine (MTA) is a sulfur-containing nucleoside produced stoichiometrically during the synthesis of the polyamines spermidine and spermine, and rapidly cleaved to adenine and 5-methythioribose-1-phosphate by a specific phosphorylase[1]. MTA phosphorylase, an enzyme exhibiting an absolute requirement for inorganic ortho-phosphate, has been partially purified from several sources[2-6]. The finding of the enzyme in most mammalian tissues explains the intracellular free adenine pool and the almost ubiquitous occurrence of the adenine phosphoribosyltransferase activity which converts adenine to purine nucleotides.

Recently, we reported the occurence of MTA phosphorylase in mammalian brain together with its development and subcellular distribution[7]. In the present communication we describe the regional distribution of the enzyme in the pig brain and some interspecies comparative investigations on the heat-resistance of the cerebral enzyme.

Whole fresh brains from pigs, cows and sheep were obtained from a local slaughterhouse. Adult rats (Wistar strain) and mice (Swiss strain) were from Morini, S. Polo d'Enza (RE), Italy, and were housed (07.00–19.00 light cycle followed by a 12 h dark cycle) in stainless steel cages (six animals per cage) for at least 48 h before sacrifice. For the regional distribution studies the brains were dissected in a cold-room over dry ice using a razor blade splint. The whole brains or the dissected cerebral areas were weighed and then homogenized in 5 vols of ice-cold 50 mM potassium phosphate buffer, pH 7.4, containing 0.3 M sucrose and 5 mM dithiothreitol. The supernatants recovered after centrifugation of the homogenates at 104,000 × g for 1 h were used as enzyme sources. MTA phosphorylase activity was determined by a previously described radiometric method[7,8] using as substrate 5′-(methyl-^{14}C)methylthioadenosine prepared by acid hydrolysis[9]. The reaction was linear with time and the amount of protein under experimental conditions used. One unit of enzyme catalyzes the formation of 1 nmol of 5-methylthioribose-1-phosphate in 60 min at 37 °C. Protein was determined by the method of Lowry et al.[10].

MTA phosphorylase distribution in pig brain was quite uniform with only moderate regional differences (table). Frontal cortex, hippocampus, thalamus and hypothalamus (spermine-rich structures) were shown to contain the highest enzymatic activity, whereas the medulla oblongata and the caudate nucleus (sper-

Specific activity and concentration of MTA phosphorylase in different areas of pig brain[a]

Area	N[b]	Specific activity (units/mg of protein)	Concentration (units/g of tissue)
Frontal cortex	4	61.6 ± 3.1	1640 ± 97
Hippocampus	4	55.7 ± 5.1	1566 ± 89
Cerebellar cortex	4	53.7 ± 3.1	1466 ± 91
Thalamus	4	53.4 ± 4.9	1326 ± 54
Hypothalamus	3	52.2 ± 4.5	1696 ± 97
Midbrain	4	44.8 ± 3.7	1521 ± 66
Pons	4	40.5 ± 0.7	1486 ± 79
Caudate nucleus	5	40.4 ± 4.5	1213 ± 59
Substantia nigra	3	36.6 ± 3.5	396 ± 44
Medulla oblongata	4	19.9 ± 0.9	835 ± 62

[a] Values are means ± SEM; [b] Number of animals.