DISTRIBUTION OF KININ-DESTROYING ACTIVITY IN BRAIN ZONES OF NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

O. P. Petrii, O. A. Gomazkov, and N. I. Vladimirova

UDC 616.12-008.331.1-092:616.831-008.94:577.175.85]-07

KEY WORDS: brain, kinin-destroying activity, peptide hydrolase, normotensive and spontaneously hypertensive rats.

Activation of the kallikrein-kinin system at the periphery and bradykinin formation are usually accompanied by lowering of the systemic blood pressure (BP). The literature on the role of kinins in the pathogenesis of cardiovascular diseases represents these physiologically active peptides as a depressor factor in regulation of the hemodynamics. The development of arterial hypertension is thus regarded as the result of disturbance of the relative function of the pressor renin-angiotensin and the depressor kinin systems of the blood [1, 3]. However, there is ever-increasing factual evidence that bradykinin, when injected intracerebrally, causes BP to rise, and not to fall [8, 11-13]. Attempts have been made to detect kinin-sensitive structures in the brain by immunofluorescence methods [4] and also to identify brain zones responsible for realization of the central hemodynamic effects of exogenously injected bradykinin [5]. There is also evidence that the principal biochemical components of the kinin system are present in the brain: kallikrein, kininogen, kinin-destroying enzyme [2, 9, 15, 18]. It can accordingly be postulated that this system, like the renin-angiotensin system of the brain [17], participates not only in the peripheral, but also in the central regulation of the hemodynamics.

This paper describes the study of changes in total kinin-destroying activity (KDA) in eight zones of the rat brain; data were obtained for the first time on the age dynamics of distribution of activity for normotensive and spontaneously hypertensive animals.

EXPERIMENTAL METHOD

The investigation was conducted on 39 male spontaneously hypertensive rats (SHR) of the Okamoto-Aoki strain and 38 normotensive rats (NTR) of the Wistar-Kyoto strain, obtained from V. V. Karpitskii at the I. M. Sechenov Yalta Research Institute of Physical Methods of Treatment and Medical Climatology. Animals of the following age groups were selected: SHR 2, 3, 6, and 12 months; NTR 3, 6, and 12 months. The weight of the rats in each age group was 180-200, 200-220, 260-280, and 300-350 g respectively. The blood pressure was measured by an indirect method without anesthesia by means of a cuff applied to the tail. The systolic pressure varied in the different groups between the following limits: in NTR aged 8-9 weeks 110-130 mm Hg, 13-14 weeks 130-150 mm Hg, 6 months 140-170 mm Hg, 12 months 140-180 mm Hg. These values correspond to age changes of BP in normotensive Wistar-Kyoto rats. For analogous groups of SHR the systolic pressure varied between the following limits: 8-9 weeks 140-160 mm Hg, 13-14 weeks 170-200 mm Hg, 6 months 210-260 mm Hg, and 12 months 190-230 mm Hg.

The brain of rats anesthetized with pentobarbital was rinsed with cold physiological saline through the carotid artery to remove blood. After decapitation of the animals the following parts of the brain were quickly removed in the cold: cerebellum, medulla, midbrain, hypothalamus, thalamus, hippocampus, striatum, and pituitary, by the method in [7]. The brain tissues were homogenized in a glass homogenizer in 10 volumes of 0.05M potassium-phosphate buffer, pH 7.5, with 0.01% Triton. The protein concentration was determined by Lowry's method. KDA was determined as follows. Since several different enzymes specifically hydrolyzing the bradykinin molecule have been identified in the brain [16], and their separate study is difficult, the method [14], whereby total KDA is estimated, was chosen. This method is based on determination of the final quantity of bradykinin by a fluorometric method after

Research Institute of Medical Enzymology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 12, pp. 649-652, December, 1985. Original article submitted February 11, 1985.

 0007-4888/85/0012-1618$09.50

TABLE 1. Changes in Total KDA (in nmoles bradykinin/mg/min) in Brain Zones of NTR and SHR of Different Ages

Part of brain	NTR			SHR			
	Aged of animals, months						
	3	6	12	2	3	6	12
Pituitary	4,37±0,04	2,17±0,40*	2,91±0,31*	3,92±0,05	4,03±0,06	3,60±0,51**	3,65±0,39
Cerebellum	2,15±0,16	1,84±0,22	2,16±0,46	1,98±0,43	1,87±0,10	2,08±0,55	2,44±0,12
Medulla	1,63±0,12	1,30±0,42	1,45±0,29	2,04±0,18	2,01±0,18	1,94±0,27	1,92±0,42
Hypothalamus	1,65±0,07	2,69±0,55	1,69±0,25	2,07±0,38	2,01±0,10	1,93±0,39	1,92±0,17
Midbrain	2,16±0,18	0,97±0,09*	1,56±0,35	2,00±0,30	2,15±0,30	1,96±0,35**	1,50±0,22
Thalamus	1,81±0,03	1,23±0,23*	1,67±0,23	2,52±0,30	2,04±0,26	2,58±0,34**	1,45±0,25
Hippocampus	2,09±0,11	1,66±0,27	2,17±0,27	2,50±0,25	2,31±0,05	2,40±0,36	2,58±0,24
Striatum	2,35±0,17	1,81±0,16*	2,04±0,20	2,68±0,35	2,37±0,24	2,42±0,35	2,63±0,22

Legend. *P ≥ 0.05 compared with original age, ** 0.05 compared with NTR.

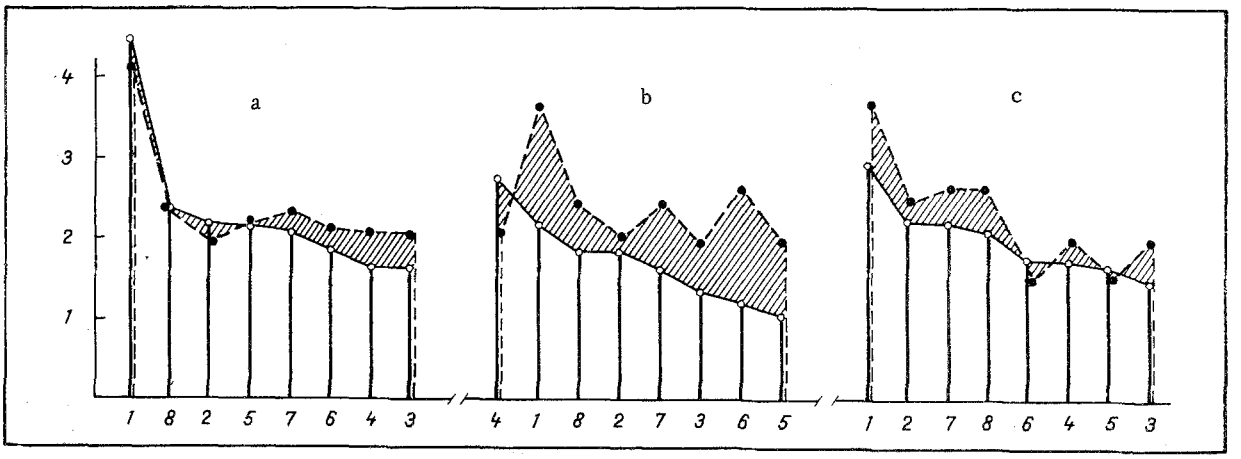

Fig. 1. Zonal distribution of KDA in brain of NTR and SHR aged 3 months (a), 6 months (b), and 12 months (c). Abscissa: 1) pituitary, 2) cerebellum, 3) medulla, 4) hypothalamus, 5) midbrain, 6) thalamus, 7) hippocampus, 8) striatum; ordinate, total KDA (in nmoles bradykinin-mg/min). Continuous line denotes NTR, broken line SHR.

separation from reaction products on a cellulose-phosphate column. The modifications introduced into the method involved an increase in volume of the cellulose phosphate used (capacity 0.9 meq/g, from Sigma, USA) by 1.5 times. Attempts were made to determine bradykinin fluorometrically by the use of o-phthalic aldehyde, which showed that bradykinin reacts weakly with this compound, and it cannot therefore be used instead of fluorescamine. KDA was determined from the decrease in the bradykinin concentrationon incubation of 20 nanomoles of bradykinin with the enzyme (200 µg protein) for 15 min at 37°C in 1 ml of 0.05M potassium-phosphate buffer, pH 7.5. Control and standard samples, not containing bradykinin or enzyme respectively, were incubated in the same way. The contents were dilute with distilled water (2 ml) and applied to a cellulose phosphate column prepared as in [6]. The column was washed successively with 3 ml of 0.03 and 0.6M sodium-phosphate buffers, pH 6.2, and 0.2M borate buffer, pH 8.5, containing 0.2M NaCl. For fluorometric determination of the bradykinin concentration, 3 ml of the borate fraction was treated with 1.0 ml of a freshly prepared solution of fluorescamine (from Serva, West Germany) in acetone (0.75 mg/ml), with vigorous mixing at room temperature. The intensity of fluorescence was measured after 10 min on an MPP-2 spectrophotometer (Hitachi, Japan) at 500 nm, with an excitation wavelength of 360 nm. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Mean values for 4-6 determinations are given in Table 1. They show that considerable changes in KDA took place in NTR aged 6 months in several brain zones. Compared with NTR aged 3 months, KDA was reduced by half in the pituitary, by 2.2 times in the midbrain, by 1.3 times in the striatum, and by 1.5 times in the thalamus. All these changes are statistically significant. KDA in the hypothalamus of NTR aged 6 months, on the other hand, showed a tendency to increase. In the other parts of the brain studied (cerebellum, medulla, hip-

pocampus) there was a tendency for KDA to fall. KDA in NTR aged 12 months returned to its initial level (3 months) in all brain zones.

The zonal investigation of KDA in NTR at successive ages thus showed that the age of 6 months stands out particularly clearly on account of a marked decline in KDA. Incidentally, in NTR at this age definite changes take place which emphasize the special role of peptides formed in the pituitary, midbrain, striatum, and thalamus.

These particular features are not found in SHR. Comparison of KDA at all ages starting with 2 months revealed no appreciable, statistically significant differences. In other words, changes in KDA characteristic of NTR at the age of 6 months were absent in SHR at the same period of development. The difference between NTR and SHR of this same age, for the pituitary, midbrain, and thalamus, was therefore 1.6, 2.0, and 2.1 times respectively. Incidentally, it is at this age that hypertension is characteristically established in the rats of the second group, Consequently, for some reason or other, a mechanism which determines the marked decrease in KDA in NTR aged 6 months is absent in SHR. Changes in KDA or NTR described above cannot be regarded as accidental, because it is a question of equivalent changes in enzyme activity characteristic of several brain zones.

For subsequent analysis of the data values of KDA in the separate brain zones of NTR were arranged in decreasing order and compared with the corresponding parameters for SHR (Fig. 1). In that way the uniformity of distribution of specific kininase activity in the brain as a whole for the different age periods could be assessed and it could be concluded that it is at the age of 6 months that the greatest falls of KDA are observed in NTR: from the highest (in the hypothalamus) to the lowest (in the midbrain). At other times (at the ages of 3 and 12 months) no significant difference was found between values of KDA in different regions of the brain. The analysis also showed that KDA in SHR aged 6 months is on the whole much higher than in NTR. Finally, it is clear that KDA in the pituitary in all cases was significantly higher than in other parts of the brain. The only exception was its value in NTR aged 6 months. These results confirm the special importance of the pituitary, and indeed of the endocrine system as a whole, in regulating the level of metabolism of the different groups of physiologically active peptides.

Previous investigations [5] were among the few attempts that have been made to identify brain structures connected with the central hemodynamic effects of kinins. One such structure, in the writers' opinion, may be the lateral septum. Similar views also were expressed in [10]. The results of the present investigation of the regional KDA in the course of development of spontaneous hypertension do not allow such a definite conclusion, i.e., no one particular part of the CNS can be specially distinguished. According to data in the literature [16], at least five different peptide hydrolases, capable of inactivating bradykinin, have been identified in the brain. They include prolyl endopeptidase, endo-oligopeptidase A, depeptidyl carboxypeptidase, enkephalinase A, etc. These enzymes may take part in metabolism of other physiologically active peptides also, such as angiotensin, enkephalins, neurotensin, substance P, etc. Our results relating to the topographic distribution of KDA in brain zones may thus have a wider interpretation for investigation of the functional activity of the various neuropeptidases under normal and pathological conditons.

LITERATURE CITED

1. O. A. Gomazkov, Vopr. Med. Khim., No. 3, 79 (1984).
2. J. Chao, S. Tanaka, and H. S. Margolius, J. Biol. Chem., 258, 6461 (1983).
3. A. M. Chernukh and O. A. Gomazkov (O. A. Gomazkow), Adv. Myocardiol., 4, 201 (1983).
4. F. M. A. Correa, R. B. Innis, G. R. Uhl, and S. H. Snyder, Proc. Natl. Acad. Sci. USA, 76, 1488 (1979).
5. F. M. A Correa and F. C. Graeff, J. Pharm. Exp. Ther., 192, 670 (1975).
6. J. Endo, J. Chromatogr., 205, 155 (1981).
7. J. Glowinski and L. L. Iversen, J. Neurochem., 13, 655 (1966).
8. F. G. Graeff, J. R. Pela, and M. Rocha e Silva, Br. J. Pharmacol., 37, 723 (1969).
9. K. Kariya, R. Kawanchi, and H. Okamoto, J. Neurochem., 36, 2086 (1981).
10. K, Kariya, Jamanchi, and J. Chatani, Neuropharmacology, 21, 267 (1982).
11. K. Kondo, T. O-Kuno, K. Konishi, et al., Naunyn Schmiederberg's Arch Pharmak., 308, 111 (1979).
12. G. A. Lambert and W. J. Lang, Eur. J. Pharmacol., 9, 383 (1970).
13. R. E. Lewis, W. E. Hoffman, and M. I. Philips, Am. J. Physiol., 244, R285 (1983).
14. E. Maita, J. Endo, and Y. Ogura, Anal. Biochem., 128, 36 (1983).

15. E. B. Oliveira, A. R. Martins, and A. C. M. Camargo, Biochemistry (Washington), 15, 1967 (1976).
16. M. Orlowski, Mol. Cell. Biochem., 52, 49 (1983).
17. M. Printz and D. Ganten, in: The Brain Renin-Angiotensin System, D. Ganten, ed., Berlin (1982), pp. 3-53.
18. T. Shikimi, R. Kema, M. Matsumoto, et al., Biochem. Pharmacol., 22, 567 (1973).

EFFECT OF AUTOLOGOUS BLOOD REINFUSION ON THE SYSTEMIC AND PORTAL CIRCULATION AFTER ACUTE BLOOD LOSS IN RATS

N. Ya. Kovalenko and D. D. Matsievskii

UDC 616-005.1-036.11-092.9-085.388-036.8-07:616.149-008.1

KEY WORDS: acute blood loss; portal macrocirculation and microcirculation; reinfusion of autologous blood.

Clinical indications for the use of autologous blood reinfusion, eliminating the risk of development of various complications connected with transplantation of homologous blood, have widened considerably at the present time [1, 5, 8]. However, the particular features of the action of autologous blood on the macro- and microcirculation in vital organs have not been adequately explained.

The aim of this investigation was to study the effect of reinfusion of autologous blood on the portal macro- and microcirculation, the systemic arterial pressure (BP), and the duration of survival of rats after acute blood loss (ABL).

EXPERIMENTAL METHOD

Experiments were carried out on 52 male Wistar albino rats weighing 200-250 g. The microcirculation in the liver and intestine was studied by contact luminescent biomicroscopy under general urethane anesthesia [7]. Simultaneously with visual inspection of the hepatointestinal microcirculation, the volume velocity of the blood flow in the portal vein of the liver and the linear velocity of the blood flow in the hepatic artery were measured by an ultrasonic method [3]. ABL was induced by one session of bleeding from the femoral artery in a volume equivalent to 2.5% of the animal's body weight in the course of 5 min. The blood was collected and stabilized with heparin, added at the rate of 2 U to 1 ml blood [5]. The heparinized autologous blood, warmed to 37°C, was reinfused after 10 min intravenously into the animal in the course of 5 min. As an integral criterion of the state of the cardiovascular system BP in the carotid artery, measured with a micromanometer, was used [6]. The duration of survival after reinfusion of autologous blood was used as an indicator of the general state of the animals.

EXPERIMENTAL RESULTS

The writers showed previously that the state of the portal macro- and microcirculation under experimental conditions correlates with the severity of the course of the posthemorrhagic period (PHP). A marked and persistent reduction of the portal fraction of the total hepatic blood flow after ABL in rats is evidence of a decompensated type of course of PHP, whereas during rapid quantitative restoration of the portal blood flow to a subnormal level PHP follows a benign course [4]. Intravenous injection of autologous blood into rats with a compensated type of course of PHP directly during reinfusion caused a rapid increase in the systemic BP and in the velocity of the hepatic portal and arterial blood flow to values 20-25% higher than initially, and an improvement of the microcirculation in the terminal vascular bed of the liver and intestine (Fig. 1). The degree of filling of the microvessels of

Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 12, pp. 652-655, December, 1985. Original article submitted January 31, 1985.

