Changes in Brain Monoamines and Their Metabolites during and after Hemorrhagic Shock in the Rat

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The alteration of monoamines and their metabolites in the brain during and after hemorrhagic shock in the conscious state was measured in rats. Blood pressure was maintained at 40–70 mmHg (5.3–9.3 kPa) for 60 min by withdrawing 8 ml of blood intermittently. The content of monoamines, as well as their metabolites, increased in various brain regions during hemorrhage, compared with the content in the control rats. Sixty min after the end of the bleeding period, almost no significant change in the contents of brain monoamines nor of their metabolites was observed. These results may indicate not only an increased release of monoamines from nerve terminals, but also an increased synthesis of them during hemorrhagic shock. Soon after the bleeding was stopped, the increased monoamine turnover rate returned to almost normal levels. (Key words: Brain, hemorrhagic shock, monoamines, rats)

(Mizobe T, Okuda C, Demura H et al.: Changes in brain monoamines and their metabolites during and after hemorrhagic shock in the rat. J Anesth 4: 116–122, 1990)

Various neurotransmitters and neuromodulators such as catecholamines[1], 5-hydroxytryptamine[2], acetylcholine[3], γ-aminobutyric acid (GABA)[4], glutamate[5] and several neuropeptides such as endorphin[6], substance-P[7] and thyrotropin-releasing hormone (TRH)[8], have been suggested to be involved in cardiovascular regulation by central nervous system. During circulatory shock and other pathophysiological conditions accompanying cardiovascular changes, the contents of some of these substances in the brain were reported to be changed[7,9].

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We previously established a reversible hemorrhagic shock model in conscious rats[10]. In this model, the reversibility of shock is predicted by the plasma lactate levels just before the animal is sacrificed for brain sampling. We have shown that the contents of TRH increased in the medulla oblongata and midbrain during hemorrhage and that the TRH contents remained at high levels in these areas and in some other brain regions 60 min after cessation of the hemorrhage. The purpose of the present study is to investigate whether the changes in brain TRH content are specific responses to the hemorrhagic shock or if there are some other neurotransmitters which respond in a similar way. Brain monoamines are known to mediate various effects in several types of stress models. In addition, brain monoamines and TRH are reported to interact in some

Table 1. Change in NE and E contents ($ng \cdot g^{-1}$ wet wt. tissue) in various brain regions during (5 min and 60 min) and after (120 min) hemorrhage

	Time (min)			
	control	5	60	120
Cerebral cortex				
NE	212 ± 30	223 ± 14	179 ± 21	174 ± 23
E	17 ± 4	10 ± 3	14 ± 2	19 ± 5
Cerebellum				
NE	164 ± 9	202 ± 22	223 ± 8*	181 ± 11
E	9 ± 2	9 ± 5	20 ± 6	10 ± 1
Midbrain				
NE	347 ± 40	345 ± 55	300 ± 56	332 ± 18
E	21 ± 6	22 ± 9	33 ± 7	19 ± 5
Medulla oblongata				
NE	446 ± 20	452 ± 15	522 ± 17*	374 ± 50
E	20 ± 6	23 ± 11	55 ± 17	15 ± 3
Striatum				
NE	660 ± 416	310 ± 66	1190 ± 517	1016 ± 511
E	34 ± 5	45 ± 22	55 ± 8	84 ± 35
Hypothalamus				
NE	855 ± 92	931 ± 98	891 ± 53	781 ± 37
E	40 ± 12	56 ± 26	183 ± 51*	75 ± 31
Hippocampus				
NE	318 ± 31	321 ± 38	181 ± 80*	216 ± 40
E	20 ± 6	28 ± 10	20 ± 6	12 ± 3

Values given are the mean ± S.E.M. (n=6; control, 5; 5min, 4; 60 min, 6; 120 min).

Significantly different compared with control, $*P < 0.05$.

brain regions[11–13]. Therefore, we measured the brain content of monoamines and their metabolites during and after hemorrhagic shock, using the same experimental model as previously reported.

Materials and Methods

Male Wistar rats weighing 180 – 200g were anesthetized with sodium pentobarbital (60 $mg \cdot kg^{-1}$ i.p.). The femoral artery was cannulated with PE 10 tubing (5 cm), the other end of which was connected to PE 50 tubing (40 cm). It was advanced subcutaneously to the posterior neck and fixed on it. After the operation, the animals were allowed to recover in individual cages (15.0 × 15.0 × 9.0 cm) for one or two days under controlled conditions (lights on at 07.00 and off at 18.00 h, temperature 24 ± 1°C), with free access to food and water. On the day of the experiment, the arterial cannula was extended outside of the cage, and the blood pressure (BP) was continuously recorded through the cannula using a pressure transducer (TMI, Model MPU-0.5-290, Tokyo) and a polygraph (San-ei, 366) calibrated with a mercury manometer. The experiments were started at 10 a.m. Hemorrhagic hypotension was produced as described previously[10]. In brief, 4 ml of blood was withdrawn through the arterial cannula at the beginning of the experiment, followed by 1 ml bleeding at 5, 15, 30 and 60 min, respectively (approximately 50% of total blood volume was withdrawn). The rats were killed by focused microwave irradiation (5 KW for 0.7 sec.) before bleeding (control) or at 5, 60, 120 min after the initial bleeding

for the measurement of cerebral monoamines and their metabolites. The blood withdrawn just before sacrifice was used to measure the plasma lactate levels. Plasma lactate levels were measured enzymatically[14] with some modifications[15].

Measurement of Cerebral Monoamines and their Metabolites

The brain was dissected on a chilled plastic plate, according to the method reported by Glowinski and Iversen[16]. Tissues were homogenized by the use of a Polytron in 0.05 M perchloric acid containing 3,4-dihydroxybenzylamine as an internal standard and centrifuged at 48000 g for 20 min at 4°C. The supernatant was stored at −80°C and was not kept longer than one week before analysis. Samples were assayed by L-4000W HPLC equipped with ODS-A reverse phase column and VMD-101A electrochemical detector (Yanaco Ltd., Kyoto, Japan) according to the method described by Wagner et al.[17] with some modifications.

Statistics

Data are presented as mean ± S.E.M. and statistical analysis was performed by one way ANOVA followed by Newman-Keuls test.

Results

The blood pressure (BP) was maintained at 40–70 mmHg (5.3–9.3 kPa) for 60 min. The plasma lactate levels just before decapitation were 1.3–1.6, 1.7–2.3 and 1.0–2.9 mM at 5, 60 and 120 min, respectively.

Norepinephrine (NE) content in the cerebellum, medulla oblongata and hippocampus, and epinephrine (E) content in the hypothalamus, showed significant increases 60 min after the initial bleeding, as compared to the control group (table 1). Statistically significant increases in dopamine (DA) contents were found in the cerebellum at 60 min and medulla oblongata at 5, 60 and 120 min. In the medulla oblongata, 3,4-dihydroxyphenylacetic acid (DOPAC) at 60 min and homovanillic acid (HVA) at 5, 60 and 120 min also showed significant increases. The ratios of (DOPAC/DA) and (HVA/DA) increased in the midbrain 120 min after the initiation of shock (table 2). There were significant increases in 5-hydroxytryptamine (5-HT) contents in the medulla oblongata at 60 min and in the hippocampus at 5 min, and in 5-hydroxyindole-3-acetic acid (5-HIAA) contents in the cerebellum, midbrain, medulla oblongata and hippocampus at 5 and/or 60 min. The ratios of (5-HIAA/5-HT) showed significant increases in the cerebral cortex, medulla oblongata, striatum and hypothalamus at 5 or 60 min (table 3).

Discussion

The change in BP at each measuring point was not significantly different compared with that in the previous report[10] which was done using the same bleeding procedure. We have established the method of predicting the prognosis of the animal after the hemorrhagic shock in the report[10]. That is, if the plasma lactate levels are under 2.9, 3.0 and 3.8 mM at 5, 60 and 120 min after the initiation of hemorrhagic shock, respectively, it can be predicted that the animals will survive 24 hr after the shock with probabilities of misdiscrimination of 32.4, 13.5 and 0.69%, at 5, 60 and 120 min, respectively. Thus, the alteration of monoamines and their metabolites, observed in this study, is considered to be measured in the reversible hemorrhagic shock model.

The present study revealed that the contents of monoamines as well as their metabolites increased in various brain regions during hemorrhagic shock (at 5 and 60 min) compared with those in the control rats. These results seem to represent not only the increased release of monoamines from the nerve terminals, but also the increased synthesis of them during the hemorrhagic period. The ratios of HVA/DA, DOPAC/DA and 5-HIAA/5-HT, which have been asserted to be good indexes of the overall turnover rate of these monoaminergic transmitters[18–20], also increased in some of the brain regions. Although we did not measure the metabolites of NE and E, a similar increase in the turnover rate may

Table 2. Changes in DA, DOPAC and HVA contents ($ng \cdot g^{-1}$ wet wt. tissue), and in the ratios of DOPAC/DA and HVA/DA

	Time (min)			
	control	5	60	120
Cerebral cortex				
DA	747 ± 72	970 ± 31	707 ± 30	818 ± 60
DOPAC	56 ± 7	72 ± 7	54 ± 10	70 ± 5
HVA	78 ± 9	83 ± 9	63 ± 8	83 ± 7
DOPAC/DA	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
HVA/DA	0.11 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Cerebellum				
DA	16 ± 9	27 ± 10	41 ± 4*	13 ± 2
DOPAC	6 ± 1	11 ± 5	9 ± 1	8 ± 2
HVA	30 ± 2	36 ± 2	42 ± 5	36 ± 4
DOPAC/DA	0.39 ± 0.09	0.40 ± 0.08	0.21 ± 0.03	0.63 ± 0.19
HVA/DA	1.86 ± 1.35	1.35 ± 0.49	1.02 ± 0.18	2.78 ± 0.25
Midbrain				
DA	192 ± 27	262 ± 30	188 ± 54	186 ± 30
DOPAC	27 ± 5	41 ± 9	29 ± 14	38 ± 6
HVA	33 ± 6	44 ± 8	43 ± 11	46 ± 5
DOPAC/DA	0.14 ± 0.01	0.16 ± 0.02	0.16 ± 0.01	0.21 ± 0.02*
HVA/DA	0.17 ± 0.01	0.18 ± 0.02	0.21 ± 0.02	0.25 ± 0.02*
Medulla oblongata				
DA	51 ± 9	88 ± 8*	97 ± 6*	81 ± 11*
DOPAC	19 ± 3	27 ± 9	45 ± 7*	31 ± 6
HVA	24 ± 2	42 ± 3**	50 ± 5**	48 ± 2**
DOPAC/DA	0.38 ± 0.04	0.31 ± 0.06	0.47 ± 0.11	0.46 ± 0.04
HVA/DA	0.49 ± 0.06	0.48 ± 0.03	0.52 ± 0.07	0.58 ± 0.06
Striatum				
DA	8560 ± 1351	9975 ± 1651	6180 ± 597	8566 ± 1013
DOPAC	546 ± 100	608 ± 83	755 ± 145	609 ± 71
HVA	495 ± 81	568 ± 47	526 ± 45	592 ± 79
DOPAC/DA	0.07 ± 0.01	0.06 ± 0.01	0.12 ± 0.07	0.07 ± 0.03
HVA/DA	0.06 ± 0.01	0.06 ± 0.01	0.08 ± 0.04	0.07 ± 0.02
Hypothalamus				
DA	809 ± 260	737 ± 385	990 ± 101	873 ± 132
DOPAC	84 ± 17	103 ± 24	170 ± 50	107 ± 18
HVA	97 ± 16	107 ± 22	115 ± 18	109 ± 7
DOPAC/DA	0.11 ± 0.01	0.14 ± 0.01	0.17 ± 0.03	0.12 ± 0.02
HVA/DA	0.12 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.13 ± 0.01
Hippocampus				
DA	463 ± 142	639 ± 94	296 ± 133	402 ± 50
DOPAC	34 ± 14	51 ± 15	21 ± 8	36 ± 4
HVA	53 ± 16	51 ± 10	27 ± 13	44 ± 4
DOPAC/DA	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.09 ± 0.01
HVA/DA	0.12 ± 0.02	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.01

Significantly different compared with control, $*P < 0.05$, $**P < 0.01$. For detail, see table 1.

Table 3. Changes in 5-HT and 5-HIAA contents ($ng \cdot g^{-1}$ wet wt. tissue), and in the ratio of 5-HIAA/5-HT

	Time (min)			
	control	5	60	120
Cerebral cortex				
5-HT	715 ± 135	886 ± 40	770 ± 149	710 ± 31
5-HIAA	236 ± 42	363 ± 21	439 ± 127	275 ± 10
5-HIAA/5-HT	0.33 ± 0.02	0.42 ± 0.02	0.56 ± 0.08**	0.38 ± 0.03
Cerebellum				
5-HT	214 ± 25	383 ± 50	392 ± 135	270 ± 41
5-HIAA	79 ± 16	174 ± 1	266 ± 61**	111 ± 7
5-HIAA/5-HT	0.38 ± 0.05	0.42 ± 0.07	0.69 ± 0.18	0.41 ± 0.05
Midbrain				
5-HT	1468 ± 248	2164 ± 206	2335 ± 832	1825 ± 207
5-HIAA	600 ± 122	1324 ± 34*	1305 ± 467	814 ± 83
5-HIAA/5-HT	0.42 ± 0.02	0.62 ± 0.06	0.56 ± 0.05	0.46 ± 0.04
Medulla oblongata				
5-HT	1134 ± 161	1615 ± 51	2421 ± 579*	1329 ± 138
5-HIAA	488 ± 65	987 ± 41*	1687 ± 411**	676 ± 86
5-HIAA/5-HT	0.44 ± 0.03	0.60 ± 0.06	0.70 ± 0.08*	0.54 ± 0.05
Striatum				
5-HT	408 ± 99	525 ± 92	323 ± 47	539 ± 196
5-HIAA	366 ± 67	573 ± 72	860 ± 152	477 ± 151
5-HIAA/5-HT	0.91 ± 0.08	1.10 ± 0.12	2.76 ± 0.96*	0.89 ± 0.23
Hypothalamus				
5-HT	756 ± 224	765 ± 285	955 ± 86	736 ± 198
5-HIAA	384 ± 111	460 ± 206	797 ± 124	360 ± 96
5-HIAA/5-HT	0.52 ± 0.02	0.60 ± 0.08	0.84 ± 0.13*	0.50 ± 0.07
Hippocampus				
5-HT	798 ± 140	1597 ± 253*	823 ± 304	937 ± 72
5-HIAA	346 ± 80	885 ± 76**	461 ± 100	423 ± 55
5-HIAA/5-HT	0.44 ± 0.01	0.56 ± 0.04	0.56 ± 0.03	0.46 ± 0.11

Significantly different compared with control, $*P < 0.05$, $**P < 0.01$. For detail, see table 1.

occur during hemorrhage. These data are consistent with other reports on the concentration of monoamines in the brain during hemorrhagic hypotension[21–23]. The different turnover patterns of DA, NE and E indicate that DA not only serves as a precursor of NE and E, but also participates differently in the functions of the central nervous system during hemorrhagic shock. At least in part, these increases in the turnover of catecholamines may be caused in order to counteract BP changes, since changes in BP do not affect the release of catecholamines in the brain after transection of the brain caudal to the hypothalamus[24]. Activation of the hypothalamic serotonergic neurons is also thought to cause the pressor effect[2]. Activation of these monoaminergic neurons, which was directly induced by hemorrhagic hypotension through the regulation center of BP, may alter functional activity in some other brain regions innervated from the cardiovascular center and may cause a series of reactions involved in the recovery from the shock. A recent study suggests that noradrenergic and dopaminergic nerves around cerebral blood vessels regulate the cerebral blood flow and prevent vasoparalysis that might be caused by perivascular tissue acidosis[25].

We must refer to the possibility that the changes in the contents of brain monoamines and their metabolites may result from brain damage due to hypotension, but we think that this is not the case. The first reason is that this shock is reversible, judging by plasma lactate levels as mentioned above. The second is that the changes in some monoamines and metabolites occur within 5 min after the onset of hypotension in some brain regions (mainly in the medulla oblongata). The third is that various compensatory responses against the hypotension are working, even 120 min after the first bleeding. For example, BP increases spontaneously after each bleeding, and arterial blood gas data are maintained within the normal range (data not shown). Thus, it is unlikely that the change in brain monoamines is caused by inhibition of the biosynthesis as a result of brain ischemia. Rather, it is probably caused by functional changes of the monoaminergic neurons in the brain which seem to be involved in various responses to shock.

It seems notable that the increases in TRH[10], as well as those in DA and 5-HT and/or their metabolites in some brain regions (mainly in the medulla oblongata), occur within 5 min after the initiation of hypotension. These results suggest that the changes in these neurotransmitters and neuromodulators occur independently and directly participate in the regulation of the cardiovascular system during the acute phase of shock. However, the possibility that a mutual interaction of catecholaminergic and TRH neurons[11–13] occurs within 5 min can not excluded.

It seems interesting that no significant changes in the contents of brain monoamines nor in their metabolites were observed 120 min after the initiation of shock (60 min after the end of shock), except for the content of DA and HVA in the medulla oblongata and the ratios of DOPAC/DA and HVA/DA in the midbrain. It is likely that the monoamine turnover rate returned to a near normal state soon after the bleeding was stopped. In the previous report, we showed that the contents of TRH increased in the medulla oblongata and midbrain during hemorrhage, and that the TRH contents remained high in these areas and in some other brain regions 120 min after the initiation of hemorrhagic shock. The functional significance of the different time courses for the turnover changes of brain monoamines and TRH, after hemorrhagic shock, is still unknown. The long-lasting increase in the TRH contents, in comparison with those of monoamines after the end of the hemorrhagic period, may be explained by several hypotheses. The first is that the increased brain TRH may represent the increased activity of TRH containing neurons. If that is true, long-lasting activation of TRH neurons may have a relation to changes such as hypovolemia and vasoconstriction, which remain even after bleeding has ceased. On the other hand, brain monoaminergic neurons seem to be activated only while the hypotension is maintained by intermittent bleeding. The second is that the difference between the TRH and monoamine contents after the shock may just reflect different pool sizes in the neurons, and the TRH neurons may no longer be activated after the shock. However, these hypotheses are difficult to confirm since TRH metabolites can not measured. Furthermore, it seems necessary to further localize the areas where the changes in monoamine turnover occur for a detailed understanding of the role of brain monoamines in hemorrhagic shock.

The experiments described in this paper were performed in adherence to the NIH guidelines for the use of experimental animals.

(Received Oct. 4, 1989, accepted for publication Dec. 12, 1989)

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