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BRIEF COMMUNICATION

Effects of DM-9384, a Pyrrolidone Derivative, on Ischemia-Induced Changes in the Central Monoamine Systems

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LUTHMAN, J., E. LINDQVIST, E. DELL'ANNA, H. KOJIMA, T. SHIOTANI, H. TACHIZAWA AND L. OLSON. *Effects of DM-9384, a pyrrolidone derivative, on ischemia-induced changes in the central monoamine systems.* PHARMACOL BIOCHEM BEHAV **41**(1) 231–234, 1992.—Alterations in brain tissue levels of monoamines and monoamine metabolites were studied in gerbils 60 min after cerebral ischemia induced by 10 min carotid ligation after pretreatment with the antiischemic drug DM-9384 (1, 3, 10, 30 mg/kg, PO). The DA levels decreased in striatum after the ischemia, while cortical and hippocampal DA levels increased. The DOPAC levels increased in cortex, but were essentially unaffected in other regions. The HVA levels increased in all forebrain regions studied. NA levels decreased in hippocampus and superior colliculus, while a general increase in MHPG levels was seen. Decreases in 5-HT levels were seen in all forebrain regions except cortex. The 10 mg/kg and 30 mg/kg doses of DM-9384 counteracted the decrease in striatal 5-HT and hypothalamic MHPG/NA ratio, respectively. Thus pretreatment with DM-9384 exerted minor protective effects on the alterations induced in monoamine systems by transient forebrain ischemia.

Ischemia Gerbils Monoamines N-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl) acetamide (DM-9384)
Dopamine Noradrenaline Serotonin

IN animal studies it has been shown that severe and prolonged brain ischemia disturbs several metabolic functions and leads to a selective pattern of cerebral infarction [e.g., (3,18)]. It has been suggested that different neurotransmitters act as contributing or protective factors in the progression of ischemia-induced infarction [see (12)]. Intracellular overload of calcium caused, at least in part, by an overactivity of excitatory amino acid systems appears to be a major pathogenic mechanism in ischemic neuronal degeneration (10,13). Enhanced release of dopamine (DA) and serotonin (5-HT) from monoaminergic neurons has also been implicated in neuronal lesions after ischemia (9,24). On the other hand, activity in the noradrenergic locus coeruleus system may limit neuronal degeneration (2). Inhibitory actions on the progression of postischemic degeneration may also be exerted by release of gamma-aminobutyric acid (GABA), taurine and adenosine (12). In line with these findings, it has been shown that drug treatments that affect various neuronal systems can influence the outcome of cerebral ischemia [e.g., (6, 10, 22)].

DM-9384 [N-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl) acetamide] is a cyclic GABA, pyrrolidone derivative, which has been shown to exert antianoxic and antiischemic actions (21,22). It has been suggested that this effect is due to an ability of DM-9384 to counteract disturbances in energy metabolism (21,22). However, it is also possible that DM-9384 affects neuronal systems that influence the outcome of cerebral ischemia, since the compound affects various parameters of GABAergic, cholinergic and monoaminergic neurotransmission (14, 16, 20). The present study was undertaken to determine whether the antiischemia effects of DM-9384 may involve effects on central monoamine systems.

METHOD

Adult gerbils (*Meriones unguiculatus*; 60–80 g, BMC, Uppsala, Sweden) were randomly divided into the different treatment groups (n = 6–7/group). Sixty minutes before the ischemia was induced, DM-9384 (Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan) was administered per os (PO, 0.5 ml) in doses of 1, 3, 10, 30 mg/kg suspended in 0.5% carboxymethylcellulose (CMC), while one group was treated with CMC only (Ischemia group). The gerbils were anesthetized with membumal (40 mg/kg, IP) and the carotids were exposed bilaterally through a ventral mid-

TABLE 1

REGIONAL CATECHOLAMINE AND CATECHOLAMINE METABOLITE LEVELS (ng/g TISSUE) AS WELL AS METABOLITE/CATECHOLAMINE RATIOS IN THE GERBIL BRAIN 1 H AFTER 10 MIN ISCHEMIA

Region and Treatment Group	DA	DOPAC	HVA	DOPA/DA	HVA/DA	NA	MHPG	MHPG/NA
Cortex								
Control	20 ± 6	8 ± <1	64 ± 9	0.56 ± 0.17	2.88 ± 0.47	198 ± 42	28 ± 5	0.16 ± 0.02
Ischemia	55 ± 9*	21 ± 3*	112 ± 12*	0.42 ± 0.06	1.81 ± 0.32	171 ± 13	75 ± 7*	0.50 ± 0.08*
10 mg/kg	47 ± 5*	16 ± 2*	106 ± 10*	0.36 ± 0.05	2.30 ± 0.15	202 ± 15	69 ± 5*	0.35 ± 0.04*
30 mg/kg	39 ± 8	19 ± 2*	99 ± 8*	0.78 ± 0.27	3.84 ± 1.32	178 ± 28	63 ± 9*	0.39 ± 0.07*
Striatum								
Control	17825 ± 655	2185 ± 167	2479 ± 195	0.12 ± 0.01	0.14 ± 0.01	136 ± 27	43 ± 5	0.38 ± 0.10
Ischemia	14964 ± 995*	2025 ± 180	3512 ± 149*	0.14 ± 0.01	0.24 ± 0.01*	150 ± 31	64 ± 8	0.60 ± 0.19*
10 mg/kg	13939 ± 981*	1924 ± 149	3345 ± 173*	0.14 ± 0.01	0.24 ± 0.02*	127 ± 45	65 ± 8	1.28 ± 0.47*
30 mg/kg	13987 ± 1607	1999 ± 205	3196 ± 227*	0.16 ± 0.04	0.24 ± 0.02*	147 ± 34	55 ± 6	0.51 ± 0.13*
Hypothalamus								
Control	637 ± 65	130 ± 12	278 ± 24	0.21 ± 0.02	0.45 ± 0.05	1360 ± 85	72 ± 9	0.06 ± 0.01
Ischemia	525 ± 70	119 ± 9	333 ± 39	0.24 ± 0.03	0.69 ± 0.12	1147 ± 93	192 ± 24*	0.17 ± 0.02*
10 mg/kg	638 ± 103	151 ± 26	435 ± 56*	0.24 ± 0.02	0.70 ± 0.05*	1385 ± 144	240 ± 53*	0.19 ± 0.06*
30 mg/kg	402 ± 48*	97 ± 7*	296 ± 36	0.25 ± 0.02	0.78 ± 0.13*	1242 ± 126	142 ± 17*	0.11 ± 0.01*†
Hippocampus								
Control	28 ± 8	14 ± 2	79 ± 6	0.54 ± 0.11	3.77 ± 0.66	231 ± 24	41 ± 2	0.19 ± 0.02
Ischemia	55 ± 8*	22 ± 3	126 ± 8*	0.40 ± 0.05	2.45 ± 0.26	150 ± 21*	75 ± 4*	0.57 ± 0.11*
10 mg/kg	54 ± 11	22 ± 3	134 ± 15*	0.46 ± 0.06	2.97 ± 0.53	155 ± 33	75 ± 6*	0.80 ± 0.33
30 mg/kg	49 ± 8	26 ± 4*	149 ± 13*	0.76 ± 0.32	3.86 ± 0.97	183 ± 27	68 ± 7*	0.42 ± 0.08
Colliculus superior								
Control	110 ± 14	18 ± 3	58 ± 3	0.16 ± 0.01	0.59 ± 0.12	352 ± 15	45 ± 3	0.13 ± 0.00
Ischemia	146 ± 11	30 ± 3*	107 ± 6*	0.20 ± 0.01*	0.75 ± 0.05	254 ± 16*	90 ± 8*	0.36 ± 0.03*
10 mg/kg	151 ± 11*	28 ± 3*	109 ± 8*	0.18 ± 0.01	0.74 ± 0.07	266 ± 24*	90 ± 10*	0.35 ± 0.04*
30 mg/kg	140 ± 23	28 ± 3*	85 ± 7*	0.35 ± 0.20	0.86 ± 0.31	244 ± 32*	84 ± 10*	0.39 ± 0.08*

DM-9384 was administered 1 h prior to the ischemia.
Data are expressed as mean ± SEM (n = 6–7).
*$p<0.05$, as compared to controls.
†$p<0.05$, as compared to the ischemia group.

line incision. Ten minute occlusions of both carotids were performed using surgical hemostats. The hemostats were thereafter removed and the animals were recirculated under continued anesthesia for 60 minutes before sacrifice. Control animals were anesthetized, the incision made, and the carotids exposed without being occluded.

The gerbils were sacrificed by decapitation and the brain tissue samples were immediately dissected out. The endogenous levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), noradrenaline (NA), 3-methoxy-4-hydroxyphenylglycol-SO_4 (MHPG), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were determined by HPLC with electrochemical detection as previously described (15).

Statistical comparisons were performed using ANOVA followed by post hoc analysis with Newman-Keuls test. The level of $p<0.05$ was considered as critical for statistical significance of differences. Metabolite/monoamine ratios were compared using the Kruskal-Wallis test. The experiments were carried out according to ethical guidelines set by the Karolinska Institute Animal Research Committee.

RESULTS

No significant effects of the ischemia were observed in pons-medulla oblongata and cerebellum, except for an increase in the DOPAC/DA ratio and the MHPG levels in pons-medulla oblongata (data not shown). Furthermore, data for the 1 and 3 mg/kg doses of DM-9384 are not presented since no specific effects were observed following these doses compared to the ischemia group.

In striatum the DA levels decreased 60 min after the 10 min period of ischemia, while in hypothalamus a tendency of decreased DA levels was seen. In cortex and hippocampus the DA levels increased, while in superior colliculus a tendency of an increase was seen. The DOPAC levels increased in cortex and superior colliculus and a tendency of increased DOPAC levels was observed in hippocampus. The HVA levels increased in cortex, striatum, hippocampus and superior colliculus, while HVA showed a tendency to increase in hypothalamus. In superior colliculus an increase in the DOPAC/DA ratio was seen and the HVA/DA ratio increased in striatum. No significant effects of pretreatment with DM-9384 were seen on the ischemia-induced DA system alterations, although at the higher doses administered a significant decrease in the DA levels was seen in superior colliculus when compared to controls. In hypothalamus a significant decrease in DOPAC and HVA as well as the HVA/DA ratio were also seen in ischemia DM-9384-pretreated rats compared to control rats. In hippocampus a significant in-

TABLE 2

REGIONAL 5-HT AND 5-HIAA LEVELS (ng/g TISSUE) AS WELL AS THE 5-HIAA/5-HT RATIO IN THE GERBIL BRAIN 1 H AFTER 10 MIN ISCHEMIA

Region and Treatment Group	5-HT	5-HIAA	5-HIAA/5-HT
Cortex			
Control	767 ± 149	134 ± 36	0.17 ± 0.04
Ischemia	796 ± 75	206 ± 18	0.26 ± 0.02
10 mg/kg	792 ± 39	191 ± 16	0.25 ± 0.03
30 mg/kg	804 ± 93	207 ± 20	0.29 ± 0.05
Striatum			
Control	566 ± 34	325 ± 37	0.57 ± 0.05
Ischemia	410 ± 19*	396 ± 20	0.97 ± 0.06*
10 mg/kg	509 ± 20†	449 ± 40*	0.90 ± 0.09*
30 mg/kg	443 ± 45	395 ± 41	0.92 ± 0.10*
Hypothalamus			
Control	1450 ± 57	384 ± 34	0.27 ± 0.03
Ischemia	895 ± 62*	386 ± 30	0.44 ± 0.04*
10 mg/kg	1155 ± 101*	460 ± 40	0.41 ± 0.03*
30 mg/kg	1054 ± 110*	374 ± 27	0.37 ± 0.04
Hippocampus			
Control	931 ± 97	273 ± 29	0.30 ± 0.02
Ischemia	541 ± 59*	323 ± 25	0.62 ± 0.05*
10 mg/kg	602 ± 73*	333 ± 17	0.59 ± 0.06*
30 mg/kg	582 ± 84*	324 ± 21	0.61 ± 0.07*
Colliculus superior			
Control	3458 ± 292	560 ± 36	0.17 ± 0.02
Ischemia	2448 ± 320*	634 ± 55	0.28 ± 0.03*
10 mg/kg	2346 ± 300*	640 ± 37	0.29 ± 0.03*
30 mg/kg	2555 ± 357	581 ± 43	0.25 ± 0.04

DM-9384 was administered 1 h prior to the ischemia.
Data are expressed as mean ± SEM (n = 6–7).
*$p<0.05$, as compared to controls.
†$p<0.05$, as compared to the ischemia group.

crease in DOPAC was observed after DM-9384 pretreatment (Table 1).

The NA tissue levels decreased in hippocampus and superior colliculus after the ischemia, while the MHPG levels increased significantly in frontal cortex, hypothalamus, hippocampus, and superior colliculus. Increases in the MHPG/NA ratio were observed in frontal cortex, hypothalamus, hippocampus and superior colliculus. The 30 mg/kg dose of DM-9384 partly counteracted the decrease of the MHPG/NA ratio in hypothalamus. Furthermore, after the DM-9384 pretreatment no significant ischemia-induced decrease was seen in the NA levels in hippocampus (Table 1).

The 5-HT levels decreased in striatum, hypothalamus, hippocampus and superior colliculus after the ischemia, while no significant alterations were seen in the 5-HIAA levels. Pretreatment with DM-9384 counteracted the ischemia-induced alterations of the striatal 5-HT levels at the 10 mg/kg dose, while after the same dose the 5-HIAA levels increased in striatum compared to control rats (Table 2).

DISCUSSION

The alterations observed in the monoamine systems are essentially in line with previously reported data from different models of partial or global ischemia (1,11). The regional differences observed in the ischemia-induced alterations of tissue monoamine levels could be due to variations in the extent of blood supply loss, e.g., major effects in forebrain regions, with minor or no effects in the brainstem (17). However, even in the forebrain regions some regionally specific changes occurred. Thus, the striatal DA tissue levels decreased 1 h after the ischemia, whereas DA increased in frontal cortex and hippocampus. NA decreased only in hippocampus and colliculus superior and 5-HT decreased in all forebrain regions except cortex. Differences in the regulation of monoamine release may be involved in these regional variations, although differences in the effects on synthesis, metabolism, as well as reuptake may contribute [see (7)].

The tissue levels of catecholamine metabolites increased in most forebrain regions, probably reflecting enhanced transmitter release during ischemia followed by enhanced metabolism during recirculation, presumably following reuptake. The decreases in the 5-HT levels may also represent increased release during ischemia, but in addition, a decrease in synthesis could be involved. Previous studies have provided indirect evidence that the monoamine oxidase (MAO) activity decreases during ischemia (19). The present results therefore indicate a rapid recovery of catecholamine reuptake and MAO functions during the recirculation period. It is, however, also possible that an ischemia-induced reduction of neuronal reuptake leads to a further increase in extracellular catecholamines and consequently enhanced extraneuronal degradation (8).

DM-9384 pretreatment did not influence the ischemia-induced effects on the DA system. This finding indicates that DM-9384 does not exert any major influence on the DA system, in agreement with a previous study on the effects of DM-9384 in rats under basal conditions (16). On the other hand, DM-9384 seems to modulate NA neurotransmission in some brain regions under both basal and ischemia conditions, since the 30 mg/kg dose partly counteracted the ischemia-induced increase in the MHPG/NA ratio in hypothalamus, and since DM-9384 also affects basal NA levels in rats (16). In striatum, the 10 mg/kg dose of DM-9384 partly counteracted the ischemia-induced 5-HT decrease, while no other effects were seen on the 5-HT system. Under basal conditions DM-9384 increases 5-HT tissue levels both acutely and chronically in rats (16). It therefore seems that the effects of DM-9384 on the 5-HT system are not restricted to basal conditions, but also occur under stressed utilization like ischemia, although species differences may be involved.

Thus under the conditions of these experiments, DM-9384 did not seem to exert any major influence on monoaminergic systems. It is known that ischemia-induced alterations in neurotransmitter levels are dependent on whether the brain ischemia is transient or sustained and if it is global or partial [e.g., (1,11)]. The use of anesthesia may also influence the effects of ischemia [see (7)]. Furthermore, temporally different alterations have been shown in monoamine transmitter levels during the reflow period (4). The method of analysis also affects the interpretation of the data, e.g., tissue or extracellular measurements (1,5). Therefore, it cannot be excluded that DM-9834 may exert a more pronounced influence on monoamine systems during ischemia under other experimental conditions. However, the present results clearly indicate that the protective actions of DM-9384 during ischemia or hypoxia are mainly related to actions on other transmitter systems, ionic alterations, or metabolic functions.

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