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Massive striatal dopamine release in acute cerebral ischemia in rats

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Summary. Extracellular dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and cerebral blood flow were simultaneously determined using in vivo brain dialysis and a hydrogen clearance method in the striatum of spontaneously hypertensive rats during ischemia and after recirculation. Massive striatal dopamine release was demonstrated in acutely induced ischemic brain.
Key words. Dopamine release; cerebral ischemia; striatum; in vivo brain dialysis; spontaneously hypertensive rats.

It has been proposed that the neurotransmitter dopamine which escapes from ischemic neurons may exacerbate the tissue damage in the striatum, probably owing to a direct effect on neurons[1], or to a hypoperfusion secondary to the dopamine-induced vasoconstriction[2]. To investigate the effects of ischemia on striatal dopamine release, in vivo brain dialysis[3] was applied to a cerebral ischemic model to determine the changes in striatal extracellular dopamine in acute cerebral ischemia.

Methods. Cerebral ischemia was induced in spontaneously hypertensive rats (SHR) by bilateral carotid artery ligation (BCL) as previously described[4, 5]. Extracellular dopamine and regional cerebral blood flow (CBF) were simultaneously determined in the striatum using in vivo brain dialysis[3] and a hydrogen clearance method[5], respectively. Five male SHR aged 5 months, weighing 300–340 g, were anesthetized with amobarbital (100 mg/kg b. wt i.p.). Both femoral arteries were cannulated; one for anaerobic sampling of blood and the other for blood pressure recording with electromanometer. Both common carotid arteries were exposed through a ventral midline incision in the neck, separated from the vagosympathetic trunks carefully, and loosely encircled with sutures for later ligation. The rat's head was fixed in a head holder, and two burr holes were made on the skull for inserting a dialysis probe and CBF electrodes. A dialysis probe, 500 μm in outer diameter (Bioanalytical System, USA) and a teflon-coated platinum electrode for CBF study, 200 μm in diameter, with a 1-mm portion at its tip uncoated, were placed stereotactically in the right striatum, 0.5 mm anterior, 2.5 mm lateral to the bregma and 4.5 mm from the brain surface. Another platinum electrode was inserted in the parietal cortex.

The striatum was perfused with a Ringer's solution of the following composition: Na^+ 147 m mol/l, Ca^{2+} 2.3, K^+ 4 and Cl^- 155.5. The solution was perfused at the rate of 1.97 μl/min with a Harvard pump. Perfusates were collected every 10 min into a plastic tube containing 5 μl of 0.2 N perchloric acid. Each 20 μl of sample was injected directly into high performance liquid chromatography with electrochemical detection (HPLC-ECD). Dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) were quantified by HPLC-ECD. The HPLC system consisted of a L-6000 pump

Arterial acid-base parameters and mean blood pressure before and during ischemia and after 60 min of recirculation

		Before	Ischemia	Recirculation
P_{CO_2}	(mm Hg)	31.8 ± 1.7	15.0 ± 1.6**	23.9 ± 1.7**
P_{O_2}	(mm Hg)	82.3 ± 1.6	95.9 ± 4.8	83.9 ± 2.9
pH		7.45 ± 0.01	7.64 ± 0.02**	7.51 ± 0.02*
MBP	(mm Hg)	200 ± 7	240 ± 6**	198 ± 7

Values are mean ± SEM (n = 5). *, $p < 0.05$; **, $p < 0.01$ vs before ischemia, paired t-test.

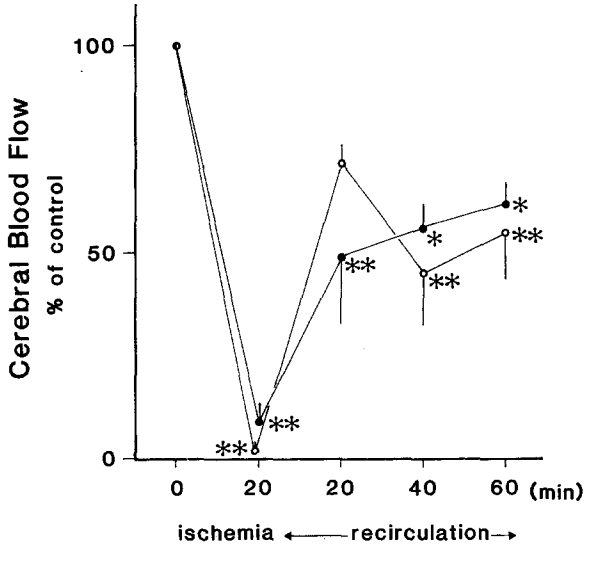

Figure 1. Percent changes in striatal (closed circle) and cortical (open circle) cerebral blood flow during ischemia and after recirculation. Bars represent SEM (n = 5). * $p < 0.05$; ** $p < 0.01$ vs before ischemia, paired t-test.

(Hitachi, Ltd., Japan) set to flow rate 1.0 ml/min, a reverse phase column (Eicompak MA-ODS, 4.6 × 250 mm, Eicom corporation, Japan) and an electrochemical detector ECD 100 (Eicom Corporation, Japan). The mobile phase was 0.1 M KH_2PO_4 (pH 4.0) containing 20 mg/l Na_2EDTA, 150 mg/l sodium octylsulphate and 18% (v/v) methanol.

After completion of the surgery, a resting period of 30 min was allowed before the experiment. Two baseline CBF and four perfusates were measured at rest and then both carotid arteries were ligated tightly for 20 min followed by recirculation for 60 min by removing the ligature bilaterally. CBF was determined every 20 min during and after cerebral ischemia. After completion of the experiment, the positions of the dialysis probe and CBF electrodes were histologically examined.

Results. Animals began to hyperventilate immediately after BCL, resulting in decreased arterial P_{CO_2} and increased pH after 20 min of ischemia. MBP rose to 240 mm Hg during ischemia but returned to the baseline level after 60-min recirculation (table). CBF markedly decreased to around 2 and 9% of the resting value in the parietal cortex and striatum, respectively, after 20-min ischemia and was partially restored to 50–60% of the resting after 60-min recirculation (fig. 1). Dopamine in the perfusate increased remarkably, to 241 times of the control, after 10-min ischemia, and returned to the control level after 30-min recirculation (fig. 2). In contrast, DOPAC decreased to 50% of the control after BCL and increased to 211% after 40 min recirculation (fig. 3).

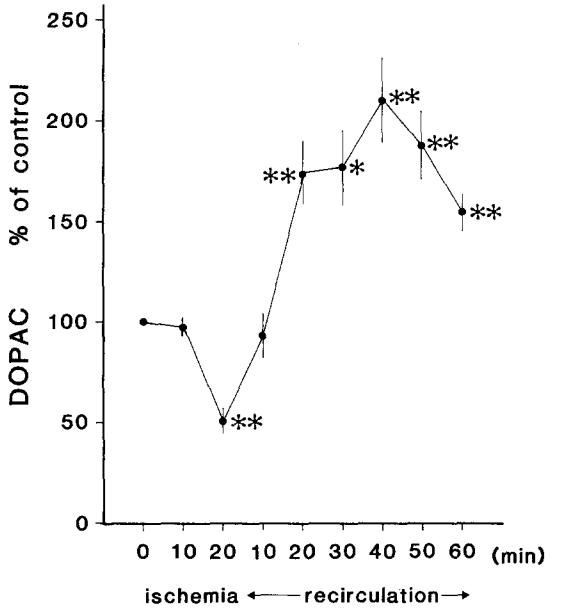

Figure 3. Changes in DOPAC in striatal perfusates collected every 10 min. The 100% value (994.9 ± 179.6 pg/20 µl) indicates the mean of the final 2-3 stable control measurements. Bars represent SEM (n = 5). *, $p < 0.05$; **, $p < 0.01$ vs before ischemia, paired t-test.

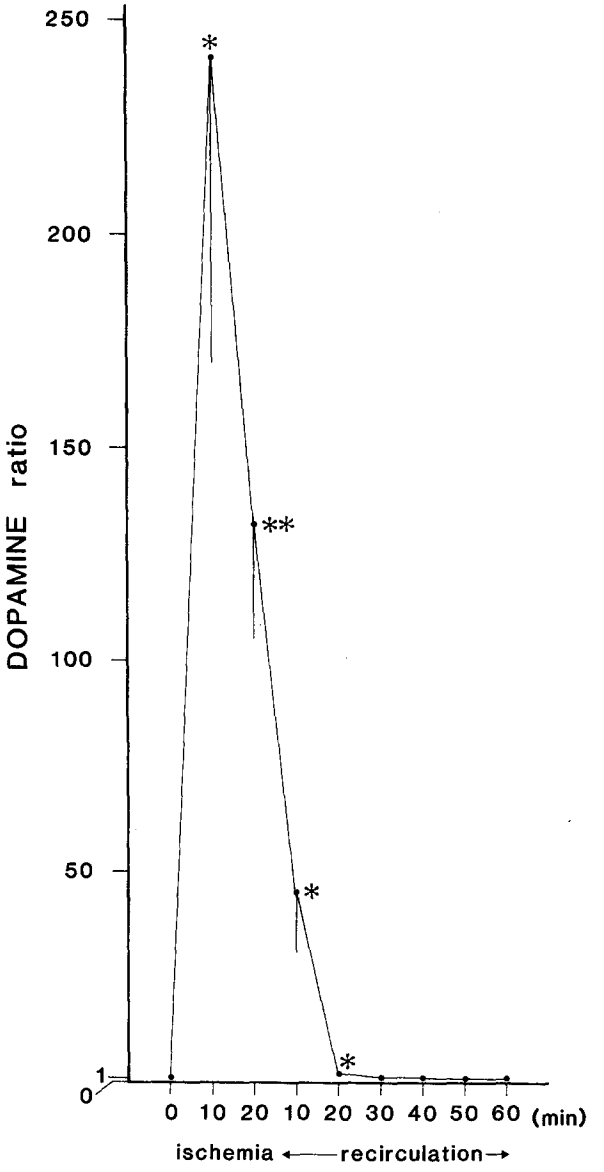

Figure 2. Changes in dopamine in striatal perfusates collected every 10 min. The 100% value (10.7 ± 0.9 pg/20 µl: dopamine ratio = 1) indicates the mean of the final 2–3 stable control measurements. Bars represent SEM (n = 5). *, $p < 0.05$; **, $p < 0.01$ vs before ischemia, paired t-test.

Discussion. We have previously reported that bilateral carotid occlusion in SHR reduces supratentorial CBF to <10 ml/100 g/min [5] and causes extensive cerebral infarction histologically [6] and biochemically [4] identical to that in humans. Using this ischemic model, we estimated dopamine release and regional CBF in the striatum simultaneously. The present study demonstrated massive dopamine release in the striatum in acute cerebral ischemia. This finding is consistent with the reported results concerning dopamine release in anoxia [7] or ischemia, which was detected by in vivo voltammetry [8]. Although the exact mechanism of this massive dopamine release is not clear from the present study, it might be caused by cell membrane dysfunction due to ischemia-induced energy failure.

It has been reported that dopaminergic activity increases during recirculation after transient ischemia [2], but striatal dopamine measured in the whole tissue changes little during ischemia, probably owing to the fact that the whole tissue measurement includes a large intracellular component. The reported 'increased dopaminergic activity' might reflect increased intracellular dopamine metabolism, synthesis and catabolism, the last of which was shown indirectly by the increased DOPAC in the perfusates during recirculation in this study.

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Brown adipose tissue activity in hypophysectomized rats: involvement of sympathetic system

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Summary. At thermal neutrality, hypophysectomy enhanced interscapular brown adipose tissue (IBAT) activity (increase of purine nucleotide binding) in the rat. This stimulation is dependent on sympathetic system integrity since surgical denervation of IBAT impairs its thermogenic response.
Key words. Hypophysectomy; sympathectomy; brown adipose tissue.

Brown adipose tissue (BAT) is the major site of both non-shivering and diet-induced thermogenesis. It has recently been found to be a common effector for both thermic and weight regulation [1]. It has been shown that the same modifications of the composition of BAT which are normally induced following cold stimulation are also observed in hypophysectomized rats acclimated at 28 °C [2]. Some enzymes known to modulate the energy supply to that organ showed enhanced activities [3] and binding of purine nucleotides in BAT mitochondria (an indicator of the thermogenic state of the tissue) was higher at thermal neutrality in hypophysectomized rats supplemented with thyroxine and corticosterone [4] or at ambient temperature [5]. The development of thermogenesis in BAT is under the control of hypothalamic thermoregulatory centers and the effector pathway involves the sympathetic nervous system via the release of norepinephrine [6], but its activity can be modified by a number of hormonal factors [7]. It remains to be demonstrated that the subsequent changes in BAT induced by hypophysectomy at thermal neutrality [2] also requires an intact sympathetic nervous system, as has been demonstrated for cold-induced stimulation [8]. In the present study the possible involvement of the sympathetic nervous system was investigated by surgical denervation of hypophysectomized rats.

Materials and methods. Male Long-Evans rats, 6–7 weeks old, were hypophysectomized by the parapharyngeal route. The effectiveness of hypophysectomy was verified by the following criteria: no weight gain, testicular atrophy, absence of pituitary remnant upon examination of the sella turcica in the sphenoid bone under binocular microscopy post mortem. The operation was simulated in another group. Only those hypophysectomized rats were used which did not gain weight over a period of at least 3 weeks. The control and hypophysectomized rats were acclimated to 28 °C for 5 weeks. 10 days before sacrifice, interscapular BAT (IBAT) of a group of hypophysectomized rats was surgically denervated: animals were anesthetized with chloral 200 mg/kg i.p., the five nerves supplying each lobe of IBAT were isolated and cut without damage to the tissue. It has already been established that such denervated IBAT is severely depleted in norepinephrine [9]. The animals were fed on a standard laboratory diet (UAR 03), manufacturer's data: 23.5% proteins, 5% lipids, 49.8% carbohydrates. All rats had free access to food and water and the lights were on from 07.00 to 19.00 h. IBAT mitochondria were prepared and purine nucleotide binding was assessed by the method described by Nedergaard et al. [10]. Various concentrations of GDP were used (0.1, 1, 3 μM) to test the linearity of a Scatchard plot and to obtain a satisfactory estimation of high affinity binding sites. The mitochondrial protein yield to IBAT mitochondria was determinated by spectrophotometric assay of cytochrome C oxidase [11] in the homogenate and in the mitochondrial fraction. Results are expressed as mean ± SEM. Statistical analysis used an unpaired t-test.

Results and discussion. Hypophysectomized rats lost some weight during the first week after operation but then maintained a stable weight of about 155 g. The weight of IBAT was significantly lower in hypophysectomized rats (214 mg vs 410 mg). However in terms of body weight (per 100 g b.wt) the amount of IBAT was the same in hypophysectomized and control rats. Hypophysectomy led to a significant decrease of 28% in the mitochondrial protein content (7.7 mg vs 10.4 mg). But when expressed per 100 g b.wt, mitochondrial proteins were significantly (50%) higher in hypophysectomized rats (4.9 mg vs 3.3 mg). Specific GDP binding (expressed per mg mitochondrial proteins) showed a significant 2.5-fold increase in the hypophysectomized rats (0.66 nmol vs 0.26 nmol). An estimate of total GDP binding per IBAT can be obtained if mitochondrial yield is combined with specific GDP binding. In this case a considerable increase of 1.8-fold was still observed for the hypophysectomized group (5.00 nmol vs 2.82 nmol).

In the hypophysectomized rat with denervated IBAT 10 days before sacrifice, body weight was the same as in hypophysectomized control rats. Expressed per 100 g body weight, denervated IBAT weight was significantly higher (165 mg vs 135 mg). Lipid accumulation explained the difference (results not shown). Total IBAT mitochondrial proteins showed a significant diminution compared to hypophysectomized rats (5.4 mg vs 7.7 mg) and to controls (5.4 mg vs 10.4 mg). In terms of body weight, mitochondrial proteins were the same in denervated IBAT of hypophysectomized rats and in normal controls. Surgical denervation completely abolished the specific GDP binding increase observed in hypophysectomized rats. Total GDP binding was five-fold lower in denervated rats than in hypophysectomized control rats (1.02 nmol vs 5.00 nmol). Expressed per 100 g body weight, total GDP binding was comparable in denervated hypophysectomized rats and in normal controls.

Our results obtained at thermal neutrality agree with Fellenz's study on hypophysectomized rats supplemented with thyroxine and corticosterone [4]. We show that even in the