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Longitudinal assessment of infarct progression, brain metabolism and behavior following anterior cerebral artery occlusion in rats

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HIGHLIGHTS

- We established an endothelin-1 model of anterior cerebral artery occlusion (ACAo).
- Autoradiography and PET disclosed transient gradual ischemia of up to 4 h.
- Comparable to abulia in humans, goal-directed executive functions deteriorated.
- In contrast, hyperactivity predominated, if task-related stimuli were absent.
- The model is well suited to study functional impairment and recovery after ACAo.

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ABSTRACT

Background: Stroke patients suffering from occlusion of the anterior cerebral artery (ACAo) develop cognitive and executive deficits. Experimental models to investigate such functional impairments and recovery are rare and not satisfyingly validated.

New method: We stereotactically injected the vasoconstrictor endothelin-1 (ET-1) close to the ACA of rats and assessed magnitude and course of CBF reduction using [^{14}C]iodoantipyrine autoradiography and [^{15}O]H_2O-PET. [^{18}F]FDG-PET and T2-weighted MRI determined regional metabolic and structural alterations. To test cognitive and executive functions, we analyzed decision-making in a food-carrying task, spatial working memory in a spontaneous alternation task and anxiety in an elevated plus maze test before and 1 month after ACAo.

Results: CBF decreased immediately after ET-1 injection, started to recover 1–2 h and returned to control 4 h thereafter. Metabolic and structural lesions developed permanently in the ACA territory. Hypometabolism occurring bilaterally in the piriform region may reflect diaschisis. Behavioral testing after ACAo revealed context-dependent changes in decision making, exploratory activity and walking speed, as well as decreased anxiety and spatial working memory.

Comparison with existing method(s): Aside from modeling a known entity of stroke patients, ACAo in rats allows to longitudinally study deterioration of cognitive and executive function without major interference by disturbed primary motor function. It complements therefore stroke research since common models using middle cerebral artery occlusion (MCAo) all affect motor function severely.

Conclusion: The established ACAo model in rats effectively reflects deficits characteristic for ACA stroke in humans. It is furthermore highly suitable for longitudinal assessment of cognitive and executive functions.

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1. Introduction

With almost 17 million strokes per year worldwide (Feigin et al., 2014), 1.3–3.0% ischemic strokes in the territory of the anterior cerebral artery (ACA) (Arboix et al., 2009; Bogousslavsky and Regli, 1990; Gacs et al., 1983; Kumral et al., 2002) lead to the considerable number of 220,000–507,000 new ACA patients annually. These patients may suffer from discrete motor dysfunction but the most prominent symptoms after ACA stroke are cognitive and executive impairments (Kumral et al., 2002; Kang and Kim, 2008; Nagaratnam et al., 1998). Thorough cognitive/executive testing has been confined to case studies (Bird et al., 2004), revealing decreased ability to perform voluntary actions (abulia) (Bogousslavsky, 1994). Abulia refers to the reduction of spontaneous speech and to Parkinson-like symptoms such as bradykinesia and hypokinesia (Kumral et al., 2002; Nagaratnam et al., 2004). More extensive cognitive analyses have been performed with patients suffering from a rupture of anterior communicating artery aneurysms, which results in ischemia analog to ACA strokes (Böttger et al., 1998; Hütter and Gilsbach, 1992; Martinaud et al., 2009). According to the mentioned studies, deficits in anterograde and retrograde memory, selective attention, task switching, planning, decision-making and concept formation are likely to occur.

Unlike experimental models of middle cerebral artery occlusion (MCAo), models of the occlusion of the anterior cerebral artery (ACAo) are only sporadically used. They may, however, serve as models not only for this specific entity of human stroke but also, in a more general sense, as models for the longitudinal study of cognitive and executive function after stroke. An advantage is that primary motor areas are supplied by the MCA, and therefore are not directly affected by ACAo. In consequence, it is possible to use behavioral tasks for the study of ischemia-induced cognitive changes without major interference by disturbed motor function. Previous behavioral experiments (simple and choice reaction time tasks) 2 and 3 weeks after ACAo suggested that motivation and attention remained intact, but executive functions were possibly impaired (Ward et al., 1998).

We first (study 1) developed and validated a rat model of ACA occlusion (ACAo) on the basis of earlier work (Ward et al., 1998) using the vasoconstrictor endothelin-1 (ET-1) to occlude the ACA. Our goal was to verify that ET-1 injection in proximity to the ACA results in a pronounced reduction of cerebral blood flow (CBF) for a time span long enough to produce ischemic damage (Hossmann, 1994). We investigated the magnitude of CBF reduction using quantitative [^{14}C]iodoantipyrine autoradiography, and the acute time course using repetitive [^{15}O]H_2O μPET.

The second goal (study 2) was to investigate executive functions longitudinally during the first month after ACAo. The main behavioral paradigm mimicked a natural foraging situation, where rats encounter food in the open and have to decide how to deal with it: either eat it at the food patch or carry it to their burrow (Takahashi and Lore, 1980). These food-handling decisions were studied in a meander maze where food was laid out, with the rat's home cage attached. Because spatial working memory and anxiety are important factors influencing decision-making in our food-carrying task, these functions were additionally investigated using a spontaneous alternation test in the Y-maze and an elevated plus maze test. Here we report ACAo-induced behavioral alterations and alterations of regional metabolic brain activity in affected brain areas.

2. Materials and methods

2.1. Animals

Experiments were carried out in accordance with the EU directive 2010/63/EU for animal experiments and the German Animal Welfare Act (TierSchG, 2006), and were approved by regional authorities (LANUV NRW). For validation of the ACAo model (study 1), 13 male Lister hooded rats (approx. 10 weeks old; Harlan, Borchen, Germany) were used to quantify CBF reduction with [^{14}C]iodoantipyrine autoradiography, and two for repetitive intraindividual CBF measurements using [^{15}O]H_2O μPET imaging. In the behavioral study (study 2), 26 rats (approx. 10 weeks old at start) were used for behavioral testing, and a subgroup of 10 rats underwent sequential PET imaging. All rats were pair-housed and maintained in an inverted 12-h light/dark cycle (lights on at 8 pm). While rats for autoradiography and repetitive CBF measurements were fed ad libitum, rats used for the behavioral study obtained a restricted diet of 80% of their free-feeding amount of food (2018 Teklad global 18% protein rodent diet; Harlan; 15–20 g) per day. Animals were weighed twice per week. Body weight was 276–324 g at the start of experiments.

2.2. Intracerebral ET-1 injection for ACA ischemia

ACAo or sham operation took place after the first sequence of behavioral testing and was performed by a single injection of ET-1 or vehicle near the pericallosal part of the ACA. Rats were anesthetized using 5% isoflurane (delivered in 70% N_2O and 30% O_2) and fixed in a stereotactic frame, where isoflurane concentration was reduced to 2.5%. Body temperature was measured with a rectal probe, and held constant at 37 °C using a heating pad. After removing skin and periost, a small hole (approx. 1 mm in diameter) was drilled midline 1.5 mm rostral from bregma, and the 26 G cannula of a Hamilton syringe was inserted 3 mm deep, measured from the level of the dura mater. ET-1 (150 pmol in 0.3 μl sterile PBS) was then injected with a rate of 0.2 μl/min in close proximity of the ACA (Fig. 1D). In sham operated animals, 0.3 μl PBS was injected. The cannula was left in place for 10 min, and then retracted. Minor bleeding from the sagittal sinus, which was pierced by the passage of the cannula, occurred in most cases. After the bleeding had stopped (after approx. 1 min), the burr hole was closed using bone wax and the skin wound was sutured followed by application of a local anesthetic (Lidocain gel).

2.3. Quantitative autoradiographic CBF measurements (study 1)

For autoradiography, 13 rats were initially anesthetized with 5% isoflurane in 70% N_2O and 30% O_2 and maintained at 2% isoflurane. Rectal temperature was kept at 37 °C using a feedback-controlled heating system. Polyethylene catheters were inserted into both femoral veins and arteries for i.v. tracer application and arterial sampling for blood gas analysis and tracer input function. Two time points were chosen: early ischemia (17–20 min; 5 ET-1 injected rats and 3 shams), and later ischemia (2 h; 3 ET-1 and 2 shams).

The [^{14}C]iodoantipyrine (IAP) technique was employed as described previously (Sakurada et al., 1978). Ten μCi/100 g body weight of [^{14}C]IAP dissolved in 1 ml of 0.9% saline (specific activity 55 mCi/mmol; Biotrend GmbH, Cologne, Germany) was applied via i.v. ramp infusion while taking arterial blood samples onto pre-weighed filter paper. After 60 s, animals were sacrificed by i.v. injection of saturated KCl solution to stop tracer delivery to the brain. Brains were removed quickly, frozen in methylbutane at −40 °C, and stored at −80 °C. Blood samples were weighed immediately after termination of experiments and placed in counting vials. A 5 ml scintillation cocktail was added, and [^{14}C]-radioactivity was measured in a scintillation counter (Wallace 1410, Pharmacia, Freiburg, Germany) using external quench correction.

Brains were cut into 20 μm cryostat sections (Leica CM3050, Leica Microsystems GmbH, Wetzlar, Germany), which were dried on a heating plate to prevent diffusion of the radioactive tracer. Sections were exposed to autoradiographic film (Hyperfilm ECL,