# MIDDLE CEREBRAL ARTERY OCCLUSION (MCAO) INDUCED BY CALCIUM IN RATS: AN ALTERNATIVE MODEL OF BRAIN ISCHEMIA

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## Introduction

Middle cerebral artery occlusion (MCAO) induced by the potent vasoconstrictor entothelin-1 (ET-1) in one of the most relevant experimental models of acute ischemic stroke in different species. ET-1 binds to $ET_A$ and $ET_B$ receptors and increases $Ca^{2+}$ influx by an activation of dihydropyridine-sensitive $Ca^{2+}$ influxes, causing a long lasting contraction of resistance vessels. ET-1 also increases the phosphorylation of miosin light chain (MLC) induced through an activation of both PKC-dependent and independent mechanisms, what in turn enhances the $Ca^{2+}$induced contraction.

On another hand, massive $Ca^{2+}$influx into hypoxic cells is a final common pathway leading to cell death in acute ischemic stroke. Animal experiments have indicated that $Ca^{2+}$ antagonists administered after cerebral ischemia are effective in reducing infarct volume and lead to improvements in neurological outcome. $Ca^{2+}$ antagonists may act as neuroprotective drugs by diminishing the influx of calcium ions through voltage-sensitive $Ca^{2+}$channels. Clinical trials with calcium antagonists suggested a beneficial effect.

Taking into account this background, we decided to explore the use of calcium instead of endothelin in order to induced MCAO, as an alternative model of brain ischemia.

## Methods

Eight male adult Wistar rats with body weights ranging from 300 to 400 g were used. They were anesthetized with intraperitoneal injection of choral hydrate (370 mg/kg), and fixed in a sterotaxic frame. The scalp was exposed after a middle line incision of the skin. A hole of 1 mm diameter was drilled 1 mm anterior to bregma and 4 mm from the middle line. After a gentle incision of dura, 2,5 µl (0.5 µl every 1 min) of saline (NaCl 0.9%) containing two concentrations of $CaCl_2{\cdot}2H_20$ (12 and 20 mg/ml, for separate experiments) was injected in the proximity of middle cerebral artery (8.7 mm depth) by means of a Hamilton syringe attached to the stereotaxic tower. The needle bebel was previously reduced to 0.25 mm long. The needle was withdrew three minutes after the last injection, and the skin was sutured. The stereotaxic atlas of Paxinos for rats was used.

After surgery, body temperature was maintained above 35 °C by placing the animals on a heating blanket at 37°C.

Rats were euthanized 24 h later using diethyl ether and brain dislocation. Brains were extracted and cut into coronal slices of 2 mm thickness , which then were put into plaques containing 0.5 % 2,3,5-triphenyltetrazolium chloride (TTC; Sigma-Aldrich), which were placed in the oven at 37°C for 20 min.
Slices were observed under stereoscope and photographed.

## Results

In most of the rats used in this study, infarct areas were observed, which were restricted to the territory supplied by the middle cerebral artery, ipsilateral to the administration of the $CaCl_2{\cdot}2H_20$ solution (see Figure 1). In both dose groups infarct areas were absent in one of the animals, due to surgical errors.

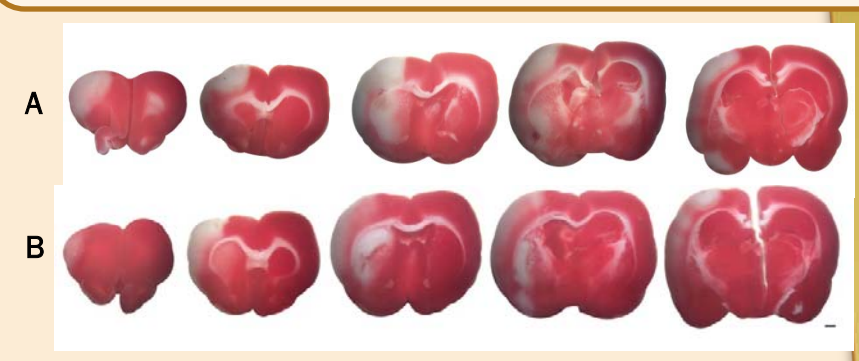


**Fig. 1. Brain slices wiht infarct areas from two rats representing the effect of intracerebral injection of $CaCl_2{\cdot}2H_20$ in the proximities (less than 0.5 mm) of the middle cerebral artery, at concentrations of 20 mg/ml (A) and 12 mg/ml (B), 24 hours after injection.**

## Conclusions

Although this is a preliminary result, it suggests that appropriate concentrations of calcium solutions can produce relatively long lasting contraction of middle cerebral artery smooth muscle as to produce experimental brain ischemia similarly to ET-1.

## References

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