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## The Effects of Morphine on Cerebral Blood Flow and its Neuroprotective or Cell Damage Before and after Brain Ischemia Reperfusion in Rabbits

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**Abstract:** Some studies have been performed on the effects of exogenous and endogenous opioids on cerebral blood flow. We investigated the effects of morphine on Cerebral Blood Flow (CBF) under normal, ischemic and reperfusion states and its neuroprotective effects after induced cerebral ischemia. Twenty male rabbits were divided in to two groups. At first CBF was measured in anesthetized rabbits as the baseline, then morphine ( $2 \text{ mg kg}^{-1}$ ) and normal saline were administered intravenously to the case and control groups, respectively. The CBF was measured again at 10, 25 and 40 min after injection. Ten minutes after injection of drugs, both common carotid vessels were occluded with microvascular clips and they were opened 25 min after injection (or 15 min after occlusion of vessels). Hippocampal subfield CA1 was studied for pathological findings of ischemia. Morphine decreased the mean CBF values at pre-ischemic stage without any changes in mean arterial blood pressure (MAP) but this reduction was not significant as compared to control group. In reperfusion stage, CBF values of both normal saline and morphine treated groups, were lower than baseline significantly ( $p < 0.05$ ), but this reduction was not significant between two groups. In assessment of neuroprotective effect and neuronal cell damage, there was not any significant difference between the two groups. Our results are in contrast with other studies which suggest morphine to have increasing or decreasing effect on CBF. This variation in results may be due to dosage and route of administration or site and method of CBF Measurement.

**Key words:** Morphine, cerebral blood flow, cell damage, neuroprotective

### INTRODUCTION

Cerebral ischemia is a major public health problem that ranks in the top four causes of death in most countries and is responsible for a large proportion of the neurological disorders (American Heart Association, 1994). Many studies have been performed on the effects of endogenous and exogenous opioids on cerebral blood flow (de Nadal *et al.*, 2000; Hoehner *et al.*, 1993; Koskinen and Bill, 1983; Ma *et al.*, 1999; Nishimura *et al.*, 1992; Zamani *et al.*, 2000). Some studies have demonstrated significant decrease in CBF (Nikolaishvili *et al.*, 2004; Nishimura *et al.*, 1992; Zamani *et al.*, 2000). But few studies have shown increased regional blood flow especially in hippocampal region, nucleus accumbens, caudate nucleus and colliculus (Koskinen and Bill, 1983; Nikolaishvili *et al.*, 2004). In other studies there is no change of CBF in most regions of the brain (de Nadal *et al.*, 2000; Hoehner *et al.*, 1993). Exogenous

and endogenous opioids can influence and modulate neuronal and cell function via an opioid receptor mediated mechanism, leading to either protection (Lim *et al.*, 2004; Yang *et al.*, 2004) or damage of the brain (Lin *et al.*, 2004). To our knowledge, there are few studies regarding morphine effects on CBP in reperfusion state and the impact of opioid administration at reperfusion is remained unclear (Peart *et al.*, 2005).

In this study, the effects of morphine on CBF was evaluated by Laser Doppler Flowmetry before and after induction of cerebral ischemia reperfusion in rabbits and also its neuroprotective effects was assessed by neuronal damage in a sensitive area of the brain to ischemia, hippocampal subfield CA1.

### MATERIALS AND METHODS

Twenty male albino rabbits weighing between 1500-3000 g were housed under diurnal light condition

with optimal access to food and water. All procedures were allowed in accordance with animal care guidance at Kerman University of Medical Science.

**Morphine administration:** Morphine sulfate 10 mg mL<sup>-1</sup> (TEMAD-IRAN) was used. It was diluted with 4 mL distilled water and was administered intravenously at dose of 2 mg kg<sup>-1</sup> with the rate of 0.5 mg min<sup>-1</sup> (Koskinen and Bill, 1983), 10 min before onset of ischemia.

**Ischemic study:** Rabbits were divided in to two groups, experimental and control groups. They were deprived of food 8-12 h before surgery. Reversible ischemia was induced by common carotid arteries occlusion in all rabbits ten minutes after receiving morphine or normal saline. The rabbits were initially anesthetized by administration of intraperitoneal ketamine hydrochloride (Rotex Media, Germany) 75 mg kg<sup>-1</sup> and xylazine (4 mg kg<sup>-1</sup>) and then after 15 min, they received thiopental sodium 50 mg kg<sup>-1</sup>. Tracheostomy was performed as artificial respiration if needed and animals were allowed to breathe spontaneously.

Maintenance dose of thiopental sodium (Biochemie, Austria) 5 mg kg<sup>-1</sup> min was administrated intravenously (Eskandary *et al.*, 2001). Rectal temperature was monitored at 37°C. In supine position right femoral artery and vein were cannulated for continuous monitoring of arterial blood pressure (physiograph, MK-III-P NARCO Biosystems USA) as well as sampling blood for measurement of pH, PaO<sub>2</sub>, PaCO<sub>2</sub> (AvL30 Acid-base Analyzer), hematocrit and blood glucose. Heparin, 500 IU kg<sup>-1</sup> was injected IV in order to prevent clotting (Koskinen and Bill, 1983).

Bilateral common carotid arteries (CCA) were exposed through a ventral midline incision. Fifteen minutes after occlusion of CCAs by temporary microvascular clips, brain became reperfused again by removal of clips (Eskandary *et al.*, 2001).

**Blood flow measurement:** After tracheostomy and cannulation of vessels, rabbit was turned to prone position and the head was fixed in a head holder. The point, 5mm posterior to bregma and 5 mm right lateral to midline was chosen for the measurement of the CBF. This point was drilled in the skull and the bone was carefully removed to prevent damage to the dura mater. Dura was opened (5×5 mm in size) and a single laser Doppler flow probe was positioned just above the cerebral cortex, normal saline was applied to moisten the cerebral cortex and fill the space between cortex and probe.

Cortical cerebral blood flow was measured by laser Doppler flow meter (Laser Doppler MBF3D Moor Instrument, England) in both groups in four stages:

- CBF-base (baseline cerebral blood flow); Measurement of CBF before administration of drug (morphine) or placebo (normal saline).
- CBF-pri (pre-ischemia cerebral blood flow); Measurement of CBF 10 min after administration of drug and before occlusion of vessels.
- CBF-end (end ischemia cerebral blood flow); Measurement of CBF 15 min after occlusion of vessels (after ischemia).
- CBF-afr (after reperfusion cerebral blood flow); Measurement of CBF 15 min after reperfusion.

**Pathological findings:** At the end of procedure the animals were deeply anesthetized and brain was fixed by infusion of 10% formalin (Nishimura *et al.*, 1992). The brain was taken out from skull and kept in formalin. The hippocampus was detached from the brain and thin longitudinal sections of hippocampus were prepared and stained with hematoxylin and eosin. The examiner was blind to Microscopic sections. The total number of neuron bodies was counted in molecular layer of CA1 region outer to hippocampal fissure and inner to stratum radiatum. With respect to the ischemic changes of CA1 region, the red neurons which are neuronal cell bodies showing hyper chromic and fragmented nuclei and deeply eosinophilic cytoplasm, were also counted in addition to intact cell bodies as a *sin qua non* feature of ischemia. Slender thin highly eosinophilic bodies without conspicuous nuclei were included in counting and were considered as artifact of fixation. The percentage of red neurons to total number of cell bodies was calculated.

**Statistical Analysis:** The CBF values were calculated as the percentage of baseline values in all stages (pre-ischemia, end-ischemia, after reperfusion in both groups. Data are expressed as mean±SEM. Paired-samples t-test and independent samples t-test was used for analysis of CBF and ischemic findings.

## RESULTS

No significant difference in physiological variables was seen between both groups (Table 1). There was not any significant difference in CBF change between morphine treated and saline treated groups, while acute and slow administration of morphine reduces CBF values in pre-ischemia and after reperfusion stages as compared to baseline ( $p < 0.05$ ) without any changes in mean blood pressure (Fig. 1). Although CBF in both pre-ischemic and reperfusion stage was reduced in normal saline treated group, but the reduction was only significant after reperfusion stage ( $p < 0.05$ ).

There was not any significant difference in pathological findings (percent of red neuron) in morphine and saline treated rabbits (Fig. 2).

Table 1: Physiological variables in normal saline (control) and morphine treated rabbits

Treatment groups	No	MAP mmHg	PaCO <sub>2</sub> mmHg	PCO <sub>2</sub> mmHg	pH	Plasma glucose mmHg	Hb g dL <sup>-1</sup>
Normal saline	10	73±9	87±3	32±4	7.2±0.3	279±34	0014.1
Morphine	10	78±6	89±4	30±2	7.3±0.4	246±21	15±2

Data are mean±SEM of 10 rabbits in each group. There was not significant difference between groups

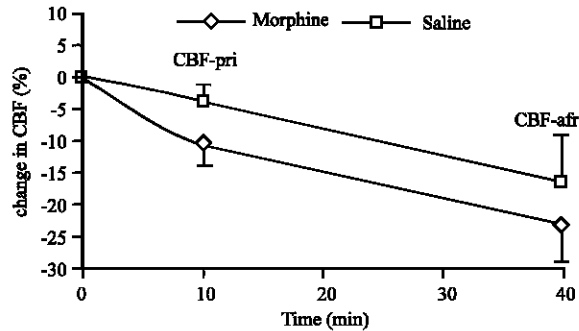


Fig 1: Percentage of mean CBF Change at two stages in morphine and saline treated rabbits. Values are expressed as means±SEM (n = 10). There was not any significant difference in CBF change between groups. pri = preischemia, afr = after reperfusion

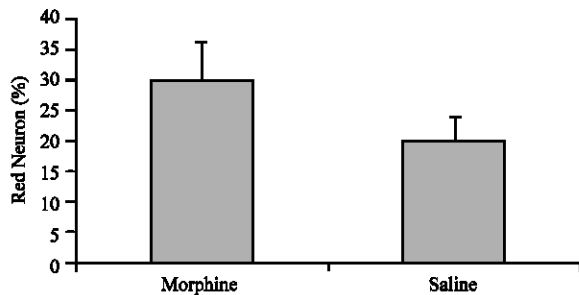


Fig 2: Percentage of neuronal cell damage (Red neuron) in both groups. Values are expressed as Mean±SEM. There was not any significant difference between groups

### DISCUSSION

**Effect of morphine on CBF:** The present study demonstrates that morphine dose not have significant effect on CBF. Present experiment was performed with a two-vessel occlusion in rabbits. This model would induce nearly complete ischemia and is useful to investigate the mechanism of ischemic neuronal cell injury (Smith *et al.*, 1984). Different effects of opioids on CBF have been reported in the literature after boluses of potent opioids given to humans and animals. To our knowledge there are a few reports on the effects of morphine on CBF, when brain is under induction of ischemia and reperfusion states. In the present study we measured CBF in these states.

Morphine (2 mg kg<sup>-1</sup>, IV) tended to increase CBF in hippocampal region, caudate nucleus and colliculus for about 30% in rabbits (Koskinen and Bill, 1983). Intraperitoneal morphine injection (1 mg kg<sup>-1</sup>) increased local blood flow in nucleus accumbens, but decreased blood flow in fronto-parietal cortex of rats (Nikolaishvili *et al.*, 2004). This result is comparable to our result, but the route of drug administration and animal model are different.

Opioids may reduce CBF by diminishing cerebral metabolic rates of oxygen or glucose (Benyo and Wahl, 1996). Endogenous opioids exert a decreasing effect on the local blood flow in the hippocampus, probably mediated by the magnocellular cholinergic neurons projecting to the hippocampus (Nishimura *et al.*, 1992). In patients with severe head injury, morphine caused significant increase in intracranial pressure and decrease in mean arterial blood pressure and cerebral perfusion pressure but estimated cerebral blood flow remained unchanged (de Nadal *et al.*, 2000). Intracarotid morphine decreased neurohypophyseal blood flow to 58% of control but it did not alter blood flow to any other brain region except caudate nucleus, in dogs (Hoehner *et al.*, 1993). In animals opiate administration has been associated with no change in CBF (McPherson and Traystman, 1984). Kirsch *et al.* (1988) have also showed that encephalinamides when administered into the cisterna magna in anesthetized dogs do not alter CBF or CMRO<sub>2</sub>, whereas they reduce neurohypophyseal blood flow. Our results also showed that morphine reduces the CBF but it was not significant.

The mechanism underlying CBF changes by morphine administration are complex and still little understood.

Morphine which enhances the action of endogenous opiates, does improve hypoxic survival in rat (Endoh *et al.*, 2001). In rats with subarachnoid hemorrhage morphine partially restores CBF autoregulation but attenuates CO<sub>2</sub>-reactivity (Ma *et al.*, 1999). It seems that endogenous opioids and central opioid receptors are involved in the cerebral cortical blood flow autoregulation and may modulate the autoregulatory vasodilatation of the cerebral cortex during the decrease in mean arterial pressure (Nagamachi *et al.*, 1995).

It is known that many parts of the brain contain enkephalin and endorphine neurons (Elde *et al.*, 1976; Johansson *et al.*, 1978) and several types of opioid receptors are distributed in distinct patterns through

central nervous system, especially in the limbic system and thalamus (Martindale, 2002). It seems likely that changes in CBF after morphine administration, reflects a change in activity of these opioid systems because there is controversy in existence of opiate receptors in the cerebral vasculature (Hoehner *et al.*, 1993; Peroutka *et al.*, 1980).

It has also been shown that the neuronal discharging patterns in different brain areas change after morphine administration (Hemstapat *et al.*, 2003; Jurna, 1981).

It seems that various brain regions were affected differently by opiate (Hoehner *et al.*, 1993; Law *et al.*, 1985; Schlaepfer *et al.*, 1998) and these different results may also be due to variation in dosage (de Nadal *et al.*, 2000; Kirsch *et al.*, 1988), method of CBF measurement (de Nadal *et al.*, 2000; Koskinen and Bill, 1983; Zamani *et al.*, 2000) and route of opioid administration (Hoehner *et al.*, 1993; Kirsch *et al.*, 1988).

The reduction of CBF after reperfusion stage was seen in both morphine and normal saline groups, thus it seems, under ischemic state some mechanisms other than opioid systems, are involved in the reduction of CBF in reperfusion stage.

**Effect of morphine on ischemic neural cell:** Our studies shows that, morphine dose not have any significant effect on ischemic neuronal cells.

Different potential mechanisms have been reported to explain neuronal cell damage and neuroprotective effects of morphine. Regulation of calcium flux has been suggested to play a role in acute and chronic effect of opioids (Lee and Yoburn, 2000). Morphine exerts its inhibitory effects presynaptically, likely through the reduction of Ca<sup>2+</sup> influx into nerve terminals and thereby inhibits the release of glutamate in the cerebral cortex. This may therefore indicate that mu-opioid receptor agonist have neuroprotective properties, especially in the excessive glutamate release that occurs under certain pathological conditions (Yang *et al.*, 2004).

Some evidences indicate that morphine may be involved in some of neuro-excitatory side effects (Lin *et al.*, 2004; Nagamachi *et al.*, 1995). Lin *et al.* (2004) have noted that morphine, which activates CaMKII via mu receptors, augments A-beta-induced LDH release, caspase-2 and caspase-3 activities and neuronal apoptosis. Morphine ineffectiveness in our study may be due to time of pathological examination and dosage of drug.

Our study demonstrates that morphine does not alter CBF and does not exert neuroprotection in ischemic reperfusion condition in rabbits (Fig. 1 and 2). The power of this study is 0.76 which shows other factors rather than

sample size might affect the results. In our experimental study there were some limitations such as dose of morphine administration and duration of ischemia that can be considered in future studies.

Another confounding factor was thiopental which reduced CBF (Veselis *et al.*, 2004), as it is seen in Fig. 2. However thiopental exerts a neuroprotective effect under ischemic condition via inhibition of NMDA receptor (Popovic *et al.*, 2000; Varathan *et al.*, 2002). However cell damage effects of CBF reduction could be concealed by neuroprotective effects of thiopental. So the remaining factor would be morphine that had not a significant effect on CBF and cell damage.

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