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BRAIN RESEARCH

Research Report

Intraventricular orexin-A improves arousal and early EEG entropy in rats after cardiac arrest

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ARTICLE INFO

Article history:
Accepted 26 November 2008
Available online 16 December 2008

Keywords:
Global ischemia
Coma
Hypocretin
Electrophysiology
Hypothalamus

ABSTRACT

The recovery of arousal after cardiac arrest (CA) is associated with evolution from electroencephalographic (EEG) burst-suppression to continuous activity. Orexin-A elicits arousal EEG during anesthetic burst-suppression. We hypothesized that orexin-A would improve arousal and EEG entropy after CA. Eighteen Wistar rats were subjected to 7-minute asphyxial CA and resuscitation. Rats were divided into treatment ($n=9$) and control ($n=9$) groups. Twenty minutes after resuscitation, the treatment group received 0.1 mL of 1 nM orexin-A intraventricularly, while controls received saline. EEG was quantified using Information Quantity (IQ), a measure of entropy validated for detection of burst-suppression and arousal patterns. IQ values range from 0 to 1.0. Arousal was quantified using the neurological deficit scale (NDS). The ischemic neuronal fraction of hippocampus CA1 and cortex was histologically determined. Baseline and post-resuscitation characteristics were similar between the groups. The NDS score (mean ± SD) at 4 h was higher in the orexin-A group compared to controls (57.3 ± 5.8 vs. 40.7 ± 5.9, $p<0.02$), but scores were similar at 72 h. Burst frequency was similar in both groups but the orexin-A group demonstrated higher IQ values compared to controls beginning within 10 min. IQ values remained significantly higher in the orexin-A group for the first 120 min ($p=0.008$) and subsequently converged. The ischemic neuronal fraction was similar between groups in cortex ($p=0.54$) and hippocampus CA1 ($p=0.14$). In rats resuscitated from CA, orexin-A transiently increased arousal and EEG entropy without worsening ischemic neuronal injury. The role of orexin-A in recovery of arousal after CA deserves further investigation.

1. Introduction

The CDC estimates that 460,000 cardiac arrests (CA) occur annually in the United States, with survival rates of 5–8% in most centers (Rosamond et al., 2008). The majority of survivors are initially comatose and disorders of consciousness represent the leading cause of chronic disability (Bedell et al., 1983; Berek et al., 1997; Vaagenes et al., 1997). Coma is presumably

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Abbreviations: EEG, electroencephalogram; CA, cardiac arrest; IQ, Information Quantity; qEEG, quantitative electroencephalogram; NDS, neurological deficit scale; H&E, hematoxylin and eosin; ROSC, return of spontaneous circulation; HDS, histological damage score; MAP, mean arterial blood pressure

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doi:10.1016/j.brainres.2008.11.102

Table 1 – Baseline characteristics of orexin and control groups

	Control (n=9)	Orexin-A (n=9)	p-value
Weight (g)	343±25	367±27	0.094
Time to CA (s)	124±26	114±27	0.446
Time to ROSC (s)	31.6±6.6	31.4±6.9	0.971
Baseline IQ	0.569±0.030	0.597±0.035	0.118
Time to bursting (min)	16.1±0.9	14.6±2.0	0.073
pH post-ROSC	7.29±0.08	7.32±0.04	0.303
PCO_2 post-ROSC (mmHg)	34.0±5.2	35.0±1.7	0.593
HCO_3 post-ROSC (mmol/L)	15.9±2.6	17.9±1.7	0.080
MAP 20 min post-ROSC (mmHg)	123.2±24.5	125.1±23.3	0.875
MAP 40 min post-ROSC (mmHg)	110.5±16.2	112.6±21.5	0.539
MAP 60 min post-ROSC (mmHg)	116.8±20.2	121.6±16.6	0.428

Time to CA is the interval between onset of asphyxia and pulse pressure <10 mmHg; time to ROSC is the interval between onset of sternal compressions and return of spontaneous circulation (ROSC); baseline IQ is the no-normalized IQ value prior to asphyxia; time to bursting is the interval between ROSC and onset of EEG burst-suppression; arterial blood gas values were determined 10 min after ROSC; MAP is mean arterial blood pressure.

due to diffuse cortical injury and selective injury to subcortical areas such as the thalamus (Longstreth, 2001; Auer and Sutherland, 2002).

Ischemic neuronal injury suppresses synaptic transmission, which is measured by changes in EEG (Holmes et al., 1983). In humans, EEG is suppressed within seconds of CA (Clute and Levy, 1990; Losasso et al., 1992; Moss and Rockoff, 1980) and recovers via an initial periodic bursting pattern that precedes restitution of continuous activity (Jorgensen and Malchow-Moller, 1981a). This transition from burst-suppression to continuous EEG precedes arousal by hours to days (Jorgensen and Malchow-Moller, 1981b). Persistent burst-suppression or failure to regain continuous EEG activity results in permanent unresponsiveness (Jorgensen and Malchow-Moller, 1981b; Scollo-Lavizzari and Bassetti, 1987). The same pattern of EEG recovery after CA has also been described in rats (Geocadin et al., 2002; Luft et al., 2002; Rosen et al., 1984), dogs (Gurvitch et al., 1972; Lin et al., 1977; Lind et al., 1975), piglets (Sherman et al., 1999), and monkeys (Myers and Yamaguchi, 1977).

In our previous work, we determined that the evolution of EEG activity after CA can be quantified using an entropy-based measure called Information Quantity (IQ) (Shin et al., 2006; Jia et al., 2006, 2008). IQ tracks recovery of EEG from low entropy states (burst-suppression) to high entropy states (desynchronized, reactive EEG). We have previously shown that rapid increase in IQ after CA is associated with faster arousal and better neurological outcomes (Jia et al., 2006, 2008).

Physiologic processes involved in resolution of EEG burst-suppression, return of continuous EEG activity, and arousal have not been studied after CA.

The hypothalamic neuropeptide orexin-A has recently emerged as a potent mediator of arousal via widespread synapses in the thalamus, cortex, and ascending brainstem networks (Eggermann et al., 2001; Marcus et al., 2001; Govidaiah and Cox, 2006; Bayer et al., 2002). Orexin deficiency has been demonstrated after global ischemia and orexin receptors are upregulated in ischemic brain (Nakamachi et al., 2005; Irving et al., 2002). Recently, intraventricular injection of orexin-A in rats with anesthetic-induced burst-suppression resulted in rapid desynchronization of EEG activity and resolution of burst-suppression (Yasuda et al., 2003; Sato-Suzuki et al., 2002; Dong et al., 2006). Orexin administration also shortens anesthetic time (Kushikata et al., 2003) whereas, inhibition of orexinergic signaling delays anesthetic emergence (Kelz et al., 2008).

Based on these observations, we hypothesized that intraventricular injection of orexin-A in rats resuscitated from CA would improve early arousal and increase EEG entropy, as quantified by IQ.

2. Results

A total of 18 rats were subjected to 7-minute asphyxial CA and resuscitation with continuous monitoring of EEG. Rats were randomly assigned to receive either 0.1 mL of 1 nM orexin-A (n=9) by intraventricular infusion or an identical volume of normal saline (n=9) 20 min after ROSC. As shown in Table 1, baseline characteristics of the 2 groups were not significantly different prior to drug delivery. There were trends for lower serum bicarbonate concentration and longer interval prior to initiation of burst-suppression in control animals but these were not statistically significant. Mean arterial blood pressure (MAP) measurements were similar just prior to orexin-A or saline infusion (20 min post-ROSC) and there were also no significant differences in MAP between the two groups after drug infusion (Table 1).

Recovery was monitored using serial NDS scores calculated by an examiner who was masked to the treatment assignment at 4, 24, 36, and 72 h after ROSC. As shown in Table 2, the mean NDS score was significantly higher at 4 and 24 h after ROSC in the orexin-A group compared to controls. NDS scores were similar between groups at 48 and 72 h. Data were reported as mean±SD at the first 2 time points because the distribution of values was normal. The other points had a left-skewed distribution of data, so NDS values were reported as median and interquartile range. The 10-point difference between groups at the 4-hour NDS measurement largely reflected a greater proportion of

Table 2 – Neurological deficit scale (NDS) in orexin-A and control groups

	Control	Orexin	p-value
4-hour NDS	44.6±6.0	54.1±5.3	0.004
24-hour NDS	62.3±6.1	70.3±3.1	0.004
48-hour NDS	70 (65–74)	74 (72–74)	0.128
72-hour NDS	72 (68–74)	74 (72–74)	0.180

4 and 24 h: mean±SD, 48 and 72 h: median (interquartile range).