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# Effects of a subconvulsive dose of kainic acid on the gene expressions of the *arginine vasopressin*, *oxytocin* and *neuronal nitric oxide synthase* in the rat hypothalamus

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ABSTRACT

Arginine vasopressin (AVP) synthesis in the hypothalamo-neurohypophysial system (HNS) is up-regulated by kainic acid (KA)-induced seizure in rats. However, it remains unknown whether a subconvulsive dose of KA affects the HNS. Here we examined the effects of subcutaneous (s.c.) administration of a low dose of KA (4 mg/kg) on the gene expressions of the *AVP*, *oxytocin* (*OXT*) and *neuronal nitric oxide synthase* (*nNOS*) in the supraoptic (SON) and paraventricular nuclei (PVN) of the rat hypothalamus, using in situ hybridization histochemistry. The expression of the *AVP* gene in the SON and PVN was judged to be up-regulated in KA-treated rats in comparison with saline-treated rats as controls. Next, the expression of the *OXT* gene was significantly increased in the SON at 6–24 h and in the PVN at 6 and 12 h after s.c. administration of KA. Finally, the expression of the *nNOS* gene was significantly increased in the SON and PVN at 3 and 6 h after s.c. administration of KA. These results suggest that up-regulation of the gene expressions of the *AVP*, *OXT* and *nNOS* in the rat hypothalamus may be differentially affected by peripheral administration of a subconvulsive dose of KA.


## 1. Introduction

The magnocellular neurosecretory cells (MNCs) in the hypothalamic supraoptic (SON) and the paraventricular nuclei (PVN) synthesize neurohypophysial hormones, arginine vasopressin (AVP) and oxytocin (OXT), project their axon terminals in the posterior pituitary and secrete AVP and OXT into the systemic circulation. The synthesis and secretion of AVP and OXT are modulated by various kinds of physiological and pathophysiological conditions, including the kainic acid (KA)-induced seizure (Iwanaga et al., 2011; Sun et al., 1996). KA is structurally related to the excitatory neurotransmitter glutamate, and binds strongly to the kainate subtype receptors as an agonist, result in excessive excitatory nerve and seizure (Collins et al., 1980). Systemic administration of KA is a good strategy to imitate the clinical and neuropathological features of temporal lobe epilepsy (Olney et al., 1974), and KA has been widely used in various kinds of seizure studies (Huang and Luijtelaar, 2012; Iwanaga et al., 2011; Ohno et al., 2012; Sakamoto et al., 2008; Turunc Bayrakdar et al., 2013). Commonly, 10–12 mg/kg of KA is systemically administered to induce seizure, which leading to massive excitotoxic damage of neuronal tissue (Doble, 1999; Iwanaga et al., 2011; Riljak et al., 2007). We demonstrated that AVP synthesis in the hypothalamo-neurohypophysial system (HNS) is up-regulated after subcutaneously (s.c.) administered 12 mg/kg kainic acid (KA)-induced seizure in rats (Iwanaga et al., 2011). On the other hand, several studies examined the effects of systemic administration of a subconvulsive or non-convulsive dose (5–6 mg/kg) of KA on the behavior in rats (Carbajal et al., 2004; Mikulecka et al., 1999; Riljak et al., 2014; Walls et al., 2014). However, up to our knowledge, there are no reports which refer to the hypothalamic neuropeptide fluctuation, in particular AVP and OXT, after systemic administration of a subconvulsive dose of KA. It has been demonstrated that systemic and central administrations of a subconvulsive dose of KA changes behavior such as locomotion,

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rearing, immobility and cognitive disturbances in rats (Arkhipov et al., 2008; Riljak et al., 2014). It is possible that a subconvulsive dose of KA could affect not only behavior but also HNS. In the present study, we investigated the effects on the gene expression of the *AVP* and *OXT* after s.c. administration of a subconvulsive dose of KA (4 mg/kg) in rats, using in situ hybridization histochemistry (ISH).

The neuronal nitric oxide synthases (nNOS) are the family of enzymes that catalyze the conversion of L-arginine to L-citrullin and nitric oxide (NO). The nNOS is abundant besides the OXT producing neurons in the hypothalamus (Nylen et al., 2001; Yamamoto et al., 1997) and it has been considered that NO produced by nNOS is one of the synthetic accelerators of OXT as well as AVP (Aguila et al., 2011; Orlando et al., 2007). Hence, we examined the effects of subconvulsive dose of KA on the gene expression of the *nNOS* in the SON and PVN as well as *AVP* and *OXT*.

## 2. Materials and methods

### 2.1. Animals

Adult male Wistar rats (170–190 g) were used for all the experiments. The animals were housed in standard plastic cages at 23–25 °C on a 12 h light (07.00–19.00 h)–12 h dark cycle. All experiments in this study were carried out in accordance with the guidelines of the Physiological Society of Japan under the control of the Ethics Committee of Animal Care and Experimentation, University of Occupational and Environmental Health, Japan.

### 2.2. Experimental procedure

Rats were s.c. administered 0.9% saline (0.5 mL/rat) as control (CTR) or KA (4 mg/kg). KA was purchased from Nacalai Tesque Inc. (Kyoto, Japan). KA was dissolved in saline and adjusted to pH 7.0. Three hours (h), 6 h, 12 h, 24 h, 48 h and 1 week after s.c. administration of saline or KA, they were decapitated without being anesthetized ($n$ = 6–7 each). After decapitation, brains were immediately removed, placed on dry ice, and stored at −80 °C in a deep freezer until use in ISH analysis.

### 2.3. Definition for a subconvulsive dose of KA

High dose of s.c. administration of KA (12 mg/kg) induced convulsion according to our previous studies (Iwanaga et al., 2011; Ohno et al., 2012). The defined seizure scale (Racine, 1972) which were rated in our previous studies exhibited around 3–4 at 3 h, the peak of the seizures, after s.c. administration of KA. In the present study every 30 min until 3 h after s.c. administration of KA (4 mg/kg)

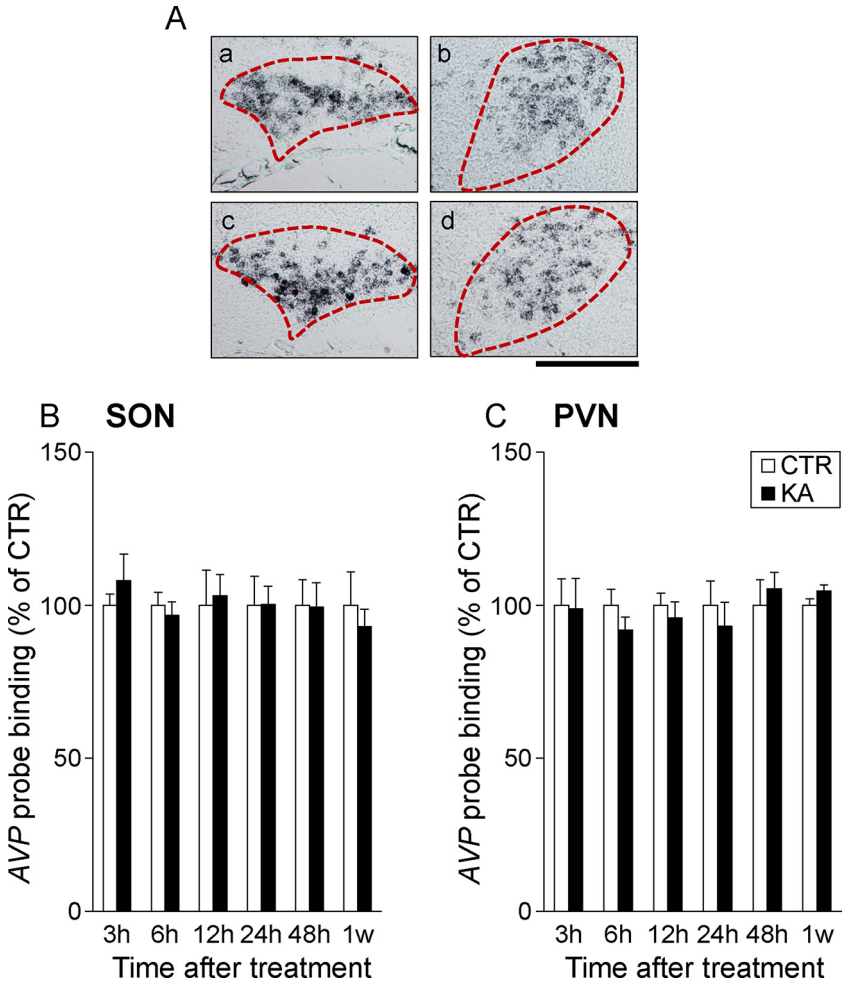


**Fig. 1.** *AVP* mRNA in the SON and PVN. (A) Representative microphotographs obtained from emulsion dipped sections that indicate *AVP* mRNA in the SON (a and c) and PVN (b and d). At 6 h after administration of saline (CTR) (a and b) or KA (c and d) in emulsion-dipped slides. Each part encircled by broken red line indicates the location used for the analysis. Scale bars, 200 μm. (B) Quantification of *AVP* signals identified in the SON. Signals for the each CTR group were set at 100%. Data are presented as means ± SEMs. CTR, $n$ = 6; KA, $n$ = 6–7. (C) Quantification of *AVP* signals identified in the PVN. Signals for the each CTR group were set at 100%. Data are presented as means ± SEMs. CTR, $n$ = 6; KA, $n$ = 6–7.