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Physiology of spontaneous $[Ca^{2+}]_i$ oscillations in the isolated vasopressin and oxytocin neurones of the rat supraoptic nucleus

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ABSTRACT

The magnocellular vasopressin (AVP) and oxytocin (OT) neurones exhibit specific electrophysiological behaviour, synthesise AVP and OT peptides and secrete them into the neurohypophysial system in response to various physiological stimulations. The activity of these neurones is regulated by the very same peptides released either somato-dendritically or when applied to supraoptic nucleus (SON) preparations *in vitro*. The AVP and OT, secreted somato-dendritically (*i.e.* in the SON proper) act through specific autoreceptors, induce distinct Ca^{2+} signals and regulate cellular events. Here, we demonstrate that about 70% of freshly isolated individual SON neurones from the adult non-transgenic or transgenic rats bearing AVP (AVP-eGFP) or OT (OT-mRFP1) markers, produce distinct spontaneous $[Ca^{2+}]_i$ oscillations. In the neurones identified (through specific fluorescence), about 80% of AVP neurones and about 60% of OT neurones exhibited these oscillations. Exposure to AVP triggered $[Ca^{2+}]_i$ oscillations in silent AVP neurones, or modified the oscillatory pattern in spontaneously active cells. Hyper- and hypo-osmotic stimuli (325 or 275 mOsmol/l) respectively intensified or inhibited spontaneous $[Ca^{2+}]_i$ dynamics. In rats dehydrated for 3 or 5 days almost 90% of neurones displayed spontaneous $[Ca^{2+}]_i$ oscillations. More than 80% of OT-mRFP1 neurones from 3 to 6-day-lactating rats were oscillatory *vs.* about 44% (OT-mRFP1 neurones) in

Abbreviations: AVP, arginine vasopressin; OT, oxytocin; MNCs, magnocellular neurosecretory cells; eGFP, enhanced green fluorescence protein; mRFP1, monomeric red fluorescence protein; SON, supraoptic nucleus; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; FFP, Fast Fluorescence Photometer; SD, Standard Deviation.

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Fura-2
Fluorescence spectrofluorimetry
Spationtemporal dynamics
Skewness

virgins. Together, these results unveil for the first time that both AVP and OT neurones maintain, via Ca^{2+} signals, their remarkable intrinsic *in vivo* physiological properties in an isolated condition.

1. Introduction

The hypothalamic supraoptic nucleus (SON) contains magnocellular neurosecretory neurones that synthesise the neurohormones arginine vasopressin (AVP) and oxytocin (OT). These hormones are released from axonal projections from these neurones to the neurohypophysis into the portal blood circulation in response to various physiological stimuli such as dehydration, osmotic stimulation, parturition and lactation. Both AVP and OT neurones exhibit characteristic electrical activities. Under physiological settings, the AVP neurones exhibit 'phasic' firing activity of action potentials with intervals, whereas OT neurones fire high frequency synchronized bursts of action potentials associated with suckling-induced milk ejection. The characteristic firing patterns, as recorded *in vivo*, are crucial for the efficient release of AVP and OT at the neurohypophysis [1,2] and from the isolated terminals [3]; and are triggered by Ca^{2+}-dependent exocytosis driven by the arrival of action potentials initiated at the cell bodies [4,5].

In the *in vivo* experiments, the somato-dendritic release of AVP modulates the phasic pattern of AVP neurones electrical activity depending on the initial firing pattern [6]. The intranuclear release of OT, which increases during suckling, increases the excitability of OT neurones [7]. At the subcellular level, AVP induces a transient increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in isolated AVP-containing neurones [8]. AVP-induced $[Ca^{2+}]_i$ responses are modulated by specific voltage-gated Ca^{2+} channel subtypes [9] and are mediated by different sub-types of AVP receptors (V_{1a}, V_{1b} and V_2) [6] through activating multiple intracellular transduction signals (PLC and AC) [10]. The autoregulation of AVP and OT neurones is mediated by distinct mechanisms: OT increases $[Ca^{2+}]_i$ in isolated OT-containing neurones through the activation of specific OT receptors and the release of Ca^{2+} from thapsigargin-sensitive intracellular stores [11,12], with subsequent activation of store-operated Ca^{2+} entry [13]. In contrast, $[Ca^{2+}]_i$ responses induced by AVP critically require plasmalemmal Ca^{2+} influx, indicating the primary role for membrane Ca^{2+} channels. Further studies have shown that the existence of a cell specific (AVP and OT) Ca^{2+} homeostasis and Ca^{2+} clearance mechanisms depends upon physiological conditions related to the specific electrical firing patterns of these neurones [14,15].

The changes in AVP release induced in response to plasma osmolarity fluctuations are mediated by regulation of action potential discharge in the cell bodies [16] and the SON neurones are defined as osmosentive [17–19]. Other studies have demonstrated that both AVP and OT neurones are activated during chronic dehydration, but with a marked difference in the pattern of their responses [20]. AVP is also shown to be involved in the promotion of water conservation during periods of dehydration [21]. Several previous studies have periodically observed that some isolated AVP and OT neurones (identified by immunocytochemistry or by their $[Ca^{2+}]_i$ responses to AVP or OT), displayed spontaneous $[Ca^{2+}]_i$ oscillations under normal experimental conditions [6,8,11,12,15,22,23]. Hitherto, however, neither the identity nor characteristics of these oscillatory neurones, nor the physiological properties of these $[Ca^{2+}]_i$ oscillations were clearly established.

In the present study, we studied $[Ca^{2+}]_i$ dynamics in i) AVP-sensitive and OT-sensitive (in terms of their specific $[Ca^{2+}]_i$ responses) neurones from wild type adult male and virgin Wistar rats; and ii) identified AVP and OT neurones from homozygous transgenic male, as well as from virgin and lactating Wistar rats models expressing (1) an arginine vasopressin (AVP)-enhanced green fluorescent protein (AVP-eGFP) [24]; (2) an oxytocin-monomeric red fluorescent protein 1 (OT-mRFP1) [25] and, (3) in homozygous double transgenic rats simultaneously bearing AVP-eGFP and OT-mRFP1 to visualize both AVP and OT neurones in the same animal [15,21,26].

2. Materials and methods

2.1. Animals and experimental procedures

Adult male Wistar rats (wild type) and three different homozygous transgenic rats were used in this study: transgenic rats expressing an arginine vasopressin-enhanced green fluorescent protein (AVP-eGFP) [25], transgenic male or female or lactating rats expressing an oxytocin-monomeric red fluorescent protein 1 (OT-mRFP1) [25] and double transgenic (expressing both markers) to visualize AVP and OT neurones [15]. A homozygous line was identified among the offspring of two heterozygous parents by finding exclusively heterozygous progeny from the mating of transgenic offspring rat with a wild-type Wistar rat. The double transgenic AVP-eGFP/OT-mRFP line was generated by mating homozygous AVP-eGFP with OT-mRFP transgenic rats. All transgenic rats used in the study were screened by polymerase chain reaction analysis of genomic DNA extracted from rat ear or tail biopsies before breeding and use in the experiments. The PCR was performed using the oligonucleotide primers (AVP-eGFP: sense sequence, 5′-CAC CAT CTT CTT CAA GGA CGA C-3′; antisense sequence, 5′-ATG ATA TAG ACG TTG TGG CTG TTG T-3′; and OT-mRFP: sense sequence, 5′-GTG AAG CAC CCC GCC GAC AT-3′; antisense sequence, 5′- TGC ATT ACG GGG CCG TCG GA-3′). The animals (weighting 150–300 g; 4–8 weeks old) were bred and housed at 22–23° C, 12:12 h light/dark cycle (lights on 07:00–19:00 h), with food and drinking water available ad libitum. The animals were sacrificed by decapitation after anaesthesia with 5% isoflurane for 5 min, the brain was rapidly removed and SON was dissected.

All animals (weighting 150–300 g; 3–8 weeks old; lactating rats weighted about 500 g; 12–16 weeks old) were bred and housed at 22–23° C under normal laboratory conditions (12:12 h light/dark cycle, lights on 07:00–19:00 h) with food and drinking water available *ad libitum*. Transgenic rats were screened by polymerase chain reaction analysis of genomic DNA extracted from rat ear or tail biopsies before breeding and use in the experiments. For each experiment, animals were sacrificed by decapitation after deep

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