Biochemistry and Biophysics Reports 7 (2016) 206–213

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Developmental change and sexual difference in synaptic modulation produced by oxytocin in rat substantia gelatinosa neurons

Chang-Yu Jiang, Tsugumi Fujita, Eiichi Kumamoto *

Department of Physiology, Saga Medical School, 5-1-1 Nabeshima, Saga 849-8501, Japan

ARTICLE INFO

Article history:
Received 4 April 2016
Received in revised form
9 June 2016
Accepted 13 June 2016
Available online 15 June 2016

Keywords:
Oxytocin
Excitatory transmission
Inhibitory transmission
Developmental change
Sexual difference
Spinal substantia gelatinosa

ABSTRACT

We have previously reported that oxytocin produces an inward current at a holding potential of −70 mV without a change in glutamatergic excitatory transmission in adult male rat spinal lamina II (substantia gelatinosa; SG) neurons that play a pivotal role in regulating nociceptive transmission. Oxytocin also enhanced GABAergic and glycinergic spontaneous inhibitory transmissions in a manner sensitive to a voltage-gated Na^+-channel blocker tetrodotoxin. These actions were mediated by oxytocin-receptor activation. Such a result was different from that obtained by other investigators in young male rat superficial dorsal horn neurons in which an oxytocin-receptor agonist enhanced glutamatergic and GABAergic but not glycinergic spontaneous transmissions. In order to know a developmental change and also sexual difference in the actions of oxytocin, we examined its effect on spontaneous synaptic transmission in adult female and young male rat SG neurons by using the whole-cell patch-clamp technique in spinal cord slices. In adult female rats, oxytocin produced an inward current at −70 mV without a change in excitatory transmission. GABAergic and glycinergic transmissions were enhanced by oxytocin, the duration of which enhancement was much shorter than in adult male rats. In young (11–21 postnatal days) male rats, oxytocin produced not only an inward but also outward current at −70 mV, and presynaptically inhibited or facilitated excitatory transmission, depending on the neurons tested; both GABAergic and glycinergic transmissions were enhanced by oxytocin. The inhibitory transmission enhancements in adult female and young male rats were sensitive to tetrodotoxin. Although the data may not be enough to be estimated, it is suggested that synaptic modulation by oxytocin in SG neurons, *i.e.*, cellular mechanism for its antinociceptive action, exhibits a developmental change and sexual difference.

1. Introduction

A posterior pituitary hormone oxytocin produces a variety of actions such as social interaction and antinociception in addition to well-known milk ejection and uterine contraction (for review see [1,2]). Although there is much evidence for an involvement of oxytocin in antinociception at the spinal cord level (for review see [3,4]), this has not yet been examined thoroughly. We have previously reported in adult (6–8 weeks old) male rats that oxytocin produces a membrane depolarization without a change in glutamatergic excitatory transmission while enhancing GABAergic and glycinergic spontaneous inhibitory transmissions in spinal lamina II (substantia gelatinosa; SG) neurons [5]. The SG neurons play a pivotal role in regulating nociceptive transmission from the periphery [6]. These oxytocin responses were mimicked by an oxytocin-receptor agonist [Thr^4, Gly^7]-oxytocin (TGOT) and inhibited by its antagonist [$d(CH_2)_5{}^1$, $Tyr(Me)^2$, Thr^4, Orn^8, des-Gly-$NH_2{}^9$]-vasotocin. The depolarization was resistant to a voltage-gated Na^+-channel blocker tetrodotoxin (TTX); the inhibitory transmission enhancements were depressed by TTX, indicating an involvement of an increase in neuronal activity [5]. On the other hand, in spinal superficial dorsal horn neurons of young (2–4 weeks old) male rats, TGOT has been reported to enhance glutamatergic and GABAergic spontaneous transmissions. Glycinergic spontaneous transmission was unaffected by TGOT [7]. Alternatively, the density of oxytocin-binding site in the superficial dorsal horn in newborn rats was higher than in adult rats [8,9]; there appeared to be a developmental change in the action of oxytocin on social interaction (for review see [10]). Thus, it is possible that oxytocin actions in the spinal dorsal horn exhibit a developmental alteration.

Abbreviations: PND, postnatal day; sEPSC, spontaneous excitatory postsynaptic current; SG, substantia gelatinosa; sIPSC, spontaneous inhibitory postsynaptic current; TGOT, [Thr^4, Gly^7]-oxytocin; TTX, tetrodotoxin; V_H, holding potential

* Corresponding author.
E-mail address: kumamote@cc.saga-u.ac.jp (E. Kumamoto).

http://dx.doi.org/10.1016/j.bbrep.2016.06.011

The role of oxytocin in social interaction exhibits sex differences. For instance, the intracerebroventricular administration of oxytocin promotes pair bonding in female but not male prairie voles ([11]; for review see [1,12]). Although there are sexual differences in the expressions of oxytocin and its receptor in the spinal dorsal horn [9], to our knowledge, it has not been examined whether oxytocin actions in the spinal dorsal horn exhibit sexual differences. In order to know a developmental change and sexual difference in the actions of oxytocin, we examined its effect on synaptic transmission in SG neurons of adult female and young male rat spinal cord slices by using the blind whole-cell patch-clamp technique and compared the results with those of adult male rats [5].

2. Materials and methods

All animal experiments were approved by the Animal Care and Use Committee of Saga University. Adult (6–8 weeks old) female and young [postnatal days (PNDs) 8–30] male rat spinal cord slice preparations were obtained in a manner similar to that described previously [5,13]. The slice was placed on a nylon mesh in the recording chamber, and was then completely submerged and superfused at a rate of 10–15 ml/min with Krebs solution which was saturated with 95% O_2/5% CO_2 and maintained at 36 ± 1 °C. The composition of Krebs solution used (in mM) was: 117 NaCl; 3.6 KCl; 2.5 $CaCl_2$; 1.2 $MgCl_2$; 1.2 NaH_2PO_4; 25 $NaHCO_3$; and 11 glucose (pH =7.4). Whole-cell voltage-clamp recordings were made from SG neurons by using patch-pipettes fabricated from thin-walled, fiber-filled capillaries, as done previously [5,13]. The patch-pipette solutions used (in mM) to record spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs, respectively) contained: 135 K-gluconate; 5 KCl; 0.5 $CaCl_2$; 2 $MgCl_2$; 5 EGTA; 5 HEPES; 5 Mg-ATP; and 110 Cs_2SO_4; 0.5 $CaCl_2$; 2 $MgCl_2$; 5 EGTA; 5 HEPES; 5 Mg-ATP; 5 tetraethylammonium-Cl (pH =7.2), respectively. The sEPSCs and sIPSCs were recorded at the holding potentials (V_Hs) of −70 and 0 mV, respectively. GABAergic and glycinergic sIPSCs were recorded in the presence of strychnine (1 μM) and bicuculline (10 μM), respectively [5,14]. Signals were acquired using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA). Data were low-pass filtered at 5 kHz, and digitized at 500 kHz with an A/D converter (Digidata 1322A, Molecular Devices). The data were stored and analyzed with a personal computer using pCLAMP 8.1 software (Molecular Devices). sEPSCs and sIPSCs were detected and analyzed using Mini Analysis Program ver. 6.0.3 (Synaptosoft, Decatur, GA, USA); detection criteria for sEPSCs or sIPSCs included a 5 pA event threshold, their fast rise time (5, 10 and 4 ms for sEPSC, GABAergic and glycinergic sIPSCs, respectively) and a decay curve (20, 20 and 10 ms for sEPSC, GABAergic and glycinergic sIPSCs, respectively) that approximated to an exponential decay [5]. When sEPSC or sIPSC frequency and amplitude changed >5% following superfusion of oxytocin, its effect was considered to be effective, as done previously [5]. When sIPSC frequency increase following oxytocin superfusion was measured in duration, the number of sIPSC events every 0.5 min in the absence and presence of oxytocin was plotted against time and then a time of period when the events increased >5% compared to those before its application was calculated. Numerical data are given as the mean ± SEM. Statistical significance was determined as $P<0.05$ using Student's t test. In all cases, n refers to the number of the neurons studied.

3. Results

3.1. Oxytocin action in adult female rat substantia gelatinosa neurons

At first, we examined the action of oxytocin (0.5 μM) on synaptic transmission in adult female rat SG neurons. Superfusing oxytocin for 3 min produced an inward current at the V_H of −70 mV, as seen in Fig. 1(A). This inward current was seen in all neurons examined, as different from adult male rat neurons, 71% of which did so ([5]; Fig. 1(B)). The peak amplitude of this current averaged to be 9.9 ± 1.1 pA ($n=8$), values comparable to those in adult male rats ([5]; Fig. 1(B)). This inward current declined in the presence of oxytocin (see Fig. 1(A)), as seen in adult male rat SG neurons [5]. On the other hand, oxytocin did not affect glutamatergic spontaneous excitatory transmission, as noted from Fig. 1 (A). The frequency and amplitude of sEPSC, measured for 0.5 min, around 1.5 min after the beginning of oxytocin superfusion were, respectively, $104 \pm 7\%$ ($P>0.05$) and $95 \pm 3\%$ ($P>0.05$) of those (control: 12.1 ± 2.6 Hz and 11.5 ± 1.6 pA; $n=8$) before its application (Fig. 1(C)).

Two kinds of GABAergic and glycinergic sIPSCs were recorded from adult female rat SG neurons, as reported previously in adult male rats (for example see [5,14]). As seen in adult male rats [5], each of the sIPSCs was enhanced in frequency and amplitude by oxytocin (0.5 μM) in all of the adult female rat SG neurons examined (see Fig. 2(A)). The enhancement in adult female rats was much shorter in duration than in adult male rats [5]. Duration times of the GABAergic and glycinergic transmission enhancements in adult female rats were 1.8 ± 0.4 min ($n=6$) and 2.3 ± 0.7 min ($n=5$), respectively. In 24 neurons where GABAergic transmission enhancement data obtained previously from adult male rats [5] were analyzed, 11 neurons exhibited a duration time of >10 min and the other neurons had an averaged duration time of 4.0 ± 0.3 min ($n=13$). These values were significantly larger than those of GABAergic transmission in adult female rats ($P<0.05$). With respect to glycinergic transmission enhancements obtained from 27 neurons [5], 13 neurons exhibited a duration time of >10 min and the other neurons had an averaged duration time of 4.4 ± 0.4 min ($n=14$). These values were significantly larger than those of glycinergic transmission in adult female rats ($P<0.05$).

At the peak of the enhancements, GABAergic sIPSC frequency and amplitude, relative to control (2.8 ± 0.7 Hz and 7.6 ± 0.3 pA; $n=6$), were, respectively, $403 \pm 52\%$ ($P<0.05$) and $172 \pm 18\%$ ($P<0.05$); corresponding glycinergic ones were, respectively, $427 \pm 110\%$ ($P<0.05$) and $223 \pm 60\%$ ($P>0.05$; $n=5$; control: 1.7 ± 0.4 Hz and 5.9 ± 1.3 pA). Each of the relative GABAergic and also glycinergic sIPSC frequency and amplitude was not significantly different in extent than that in adult male rats [GABAergic sIPSC frequency and amplitude: $538 \pm 81\%$ ($n=24$) and $166 \pm 9\%$ ($n=20$), respectively; glycinergic sIPSC frequency and amplitude: $678 \pm 70\%$ ($n=27$) and $135 \pm 7\%$ ($n=19$), respectively] which was measured around 2 min after the onset of oxytocin superfusion [5] ($P>0.05$), except for glycinergic sIPSC amplitude. A significant difference in the amplitude may be due to the fact that the inhibitory transmission enhancement is due to not a direct action of oxytocin but its depolarizing effect leading to the production of action potentials (see [5]).

The facilitatory effects of oxytocin on GABAergic and glycinergic spontaneous transmissions in adult female rat SG neurons disappeared in the presence of TTX (0.5 μM; Fig. 2(B)). Under the pretreatment with TTX for 4 min, GABAergic sIPSC frequency and amplitude, measured for 0.5 min, around 1 min after the onset of oxytocin superfusion, relative to those just before its superfusion in the presence of TTX (2.4 ± 1.4 Hz and 7.2 ± 0.4 pA; $n=5$), were, respectively, $110 \pm 10\%$ ($P>0.05$) and $96 \pm 6\%$ ($P>0.05$); corresponding glycinergic ones were, respectively, $114 \pm 6\%$ ($P>0.05$) and $99 \pm 7\%$ ($P>0.05$; $n=4$; control: 1.0 ± 0.3 Hz and 10.1 ± 1.0 pA). These results indicate an involvement of an increase in neuronal activity in the facilitations, as seen in adult male rats [5].