Synaptic modulation and inward current produced by oxytocin in substantia gelatinosa neurons of adult rat spinal cord slices

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Jiang CY, Fujita T, Kumamoto E. Synaptic modulation and inward current produced by oxytocin in substantia gelatinosa neurons of adult rat spinal cord slices. J Neurophysiol 111: 991–1007, 2014. First published December 11, 2013; doi:10.1152/jn.00609.2013.—Cellular mechanisms for antinociception produced by oxytocin in the spinal dorsal horn have not yet been investigated thoroughly. We examined how oxytocin affects synaptic transmission in substantia gelatinosa neurons, which play a pivotal role in regulating nociceptive transmission, by applying the whole-cell patch-clamp technique to the substantia gelatinosa neurons of adult rat spinal cord slices. Bath-applied oxytocin did not affect glutamatergic spontaneous, monosynaptically-evoked primary-afferent Aδ-fiber and C-fiber excitatory transmissions. On the other hand, oxytocin produced an inward current at −70 mV and enhanced GABAergic and glycinergic spontaneous inhibitory transmissions. These activities were repeated with a slow recovery from desensitization, concentration-dependent and mimicked by oxytocin-receptor agonist. The oxytocin current was inhibited by oxytocin-receptor antagonist, intracellular GDPβS, U-73122, 2-aminoethoxydiphenyl borate, but not dantrolene, chelerythrine, dibutyryl cyclic-AMP, CNQX, Ca2+-free and tetrodotoxin, while the spontaneous inhibitory transmission enhancements were depressed by tetrodotoxin. Current-voltage relation for the oxytocin current reversed at negative potentials more than the equilibrium potential for K\(^+\), or around 0 mV. The oxytocin current was depressed in high-K\(^+\), low-Na\(^+\) or Ba\(^2+\)-containing solution. Vasopressin V\(_{1A}\)-receptor antagonist inhibited the oxytocin current, but there was no correlation in amplitude between a vasopressin-receptor agonist [Arg\(^8\)]vasopressin and oxytocin responses. It is concluded that oxytocin produces a membrane depolarization mediated by oxytocin but not vasopressin-V\(_{1A}\) receptors, which increases neuronal activity, resulting in the enhancement of inhibitory transmission, a possible mechanism for antinociception. This depolarization is due to a change in membrane permeabilities to K\(^+\) and Na\(^+\), which is possibly mediated by phospholipase C and inositol 1,4,5-triphosphate-induced Ca\(^{2+}\)-release.

oxytocin; spinal dorsal horn; patch clamp; pain

A POSTERIOR PITUITARY HORMONE, oxytocin, has been recently demonstrated to have various actions, including learning and memory, social interaction and antinociception, other than milk ejection during lactation and uterine contraction during parturition (Gimpl and Fahrenholz 2001; Lee et al. 2009; Raggenbass 2001; Stoop 2012). There is much evidence showing that oxytocin inhibits primary-afferent evoked excitatory transmission in substantia gelatinosa neurons and reduce the release of L-glutamate onto SG neurons (Fürst 1999). The SG neurons receive not only excitatory but also inhibitory transmission (Willis and Coggeshall 1991), the modulation of which may also play a role in regulating nociceptive transmission (Coulth 2003; Moore et al. 2002; for review see Kohno 2007). Breton et al. (2008) have reported in spinal superficial dorsal horn neurons of young (2–4 wk old) rats that an oxytocin-receptor agonist increases the spontaneous release of L-glutamate on GABAergic interneurons, resulting in GABA release enhancement, a cellular mechanism for antinociception produced by oxytocin. Although an L-glutamate release increase produced by oxytocin has been reported in neonate rat spinal superficial dorsal horn neurons in culture (Jo et al. 1998), Robinson et al. (2002) have demonstrated that oxytocin inhibits primary-afferent evoked excitatory transmission in adult mouse spinal superficial dorsal horn neurons by activating oxytocin receptors. On the other hand, Schorscher-Petcu et al. (2010) have reported that antinociception produced by systemically administrated oxytocin is mediated by vasopressin V\(_{1A}\) but not oxytocin receptors in the mouse spinal dorsal horn. Thus it remains to be examined how oxytocin affects synaptic transmission in the SG neurons. To know a role of oxytocin in regulating nociceptive transmission, we examined its effects on holding currents, excitatory and (GABAergic and glycinergic) inhibitory transmissions by applying the blind whole-cell patch-clamp technique to the SG neurons of adult rat spinal cord slices.
METHODS

All animal experiments were approved by the Animal Care and Use Committee of Saga University.

Slice preparation. Adult rat spinal cord slice preparations were obtained in a manner similar to that described previously (Jiang et al. 2009). In brief, adult male Sprague-Dawley rats (6–8 wk old; 200–300 g) were anesthetized with urethane (1.5 g/kg body wt ip) and a laminectomy was performed to extract a lumbarosacral spinal cord enlargement (L1–S3). The spinal cord was carefully removed in a manner such that blood did not have a detrimental effect on the tissue and was then quickly immersed in ice-cold (1–3°C) Krebs solution (in mM: NaCl 117, KCl 3.6, CaCl2 2.5, MgCl2 0.5, NaH2PO4 1.2, NaHCO3 25 and glucose 11) bubbled 95% O2/5% CO2. Rats were killed by exsanguination. A transverse slice (650–700 μm thick) without or with a dorsal root was cut using a microclicer (DTK-1000, Dousaka, Kyoto, Japan) in oxygenated ice-cold Krebs solution. The slice was placed on a nylon mesh in the recording chamber (volume: 0.5 ml) and then perfused at a rate of 10–15 ml/min with Krebs solution bubbled with 95% O2 and 5% CO2, and maintained at 36 ± 1°C. The Krebs solution contained NaCl 177, KCl 3.6, CaCl2 2.5, MgCl2 1.2, NaH2PO4 1.2, NaHCO3 25 and glucose 11 (in mM; pH = 7.4 when saturated with the gas).

Whole-cell voltage-clamp recordings. The SG was identified as a translucent band under a binocular microscope with light transmitted from below, as shown previously (Jiang et al. 2009). Blind whole-cell voltage-clamp recordings were performed from neurons located at the center of SG to avoid recordings from laminae I and III neurons. Patch-pipettes were fabricated from thin-walled, fiber-filled capillaries (1.5 mm outer diameter) and contained the following solution (in mM): K-glucuronate 135, KCl 5, CaCl2 0.5, MgCl2 2, EGTA 5, Mg-ATP 5, tetraethylammonium (TEA)-Cl 5 (pH 7.2). The former and latter solutions were used to record excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs, respectively), respectively. The patch-pipettes had a resistance of 8–18 MΩ. Although the patch-pipette solution did not include GTP, we had not noted a rundown of G protein-coupled receptors, such as oxytocin receptors, as seen for opioid receptor-like-1, adenosine A1, μ-opioid and galanin receptors (Fujita and Kumamoto 2006; Liu et al. 2004; Luo et al. 2001; Yue et al. 2011). This is possibly due to a very high patch-pipette resistance and, therefore, preventing washout of the intracellular GTP during the whole-cell mode. EPSCs were recorded at a holding potential (Vh) of − 70 mV, where no IPSCs were observed, since the reversal potential for IPSCs was near − 50 mV. On the other hand, IPSCs were observed at a Vh of 0 mV, where EPSCs were invisible owing to the reversal potential for EPSCs to be close to 0 mV. Cs+ and TEA were added to inhibit K+ channels located in the recorded SG neurons and thus to easily shift Vh to 0 mV from resting membrane potentials.

Aδ-fiber- and C-fiber-evoked EPSCs were elicited by stimulating the dorsal root, as mentioned previously (Ataka et al. 2000; Nakatsuka et al. 2000). In brief, the stimulation was performed by using a suction electrode with a constant current source of pulse at a frequency of 0.1 Hz, unless otherwise mentioned. The strength of the stimuli (duration: 0.1 ms) was used 1.2 times the threshold to elicit EPSCs, fearing a conduction block of action potentials in the dorsal root. These evoked EPSCs were distinguished from each other, based on minimal stimulus strength, enough to elicit the EPSCs, and a latency of the EPSCs. C-fiber EPSCs required much larger stimulus intensity for the activation than Aδ-fiber EPSCs and had a longer latency than Aδ-fiber EPSCs. Aδ-fiber EPSCs were judged to be monosynaptic when the latency remained constant, and there was no failure during stimulation at 20 Hz for 1 s, while C-fiber EPSCs were monosynaptic when failures did not occur during repetitive stimulation at 1 Hz for 20 s, as shown previously (Ataka et al. 2000; Nakatsuka et al. 2000). The latter identification was based on the properties of the C-fiber action potentials examined by applying the sharp glass-microelectrode technique to rat dorsal root ganglion (DRG) neurons, with a repeated stimulation at 1 Hz for 20 s. The C-fiber action potential latency had a tendency to be variable, while there was no failure (see Ataka et al. 2000). Conduction velocities (CVs) of the afferent fibers were calculated from the latency of monosynaptic EPSC and the length of the dorsal root (see Ataka et al. 2000; Nakatsuka et al. 2000).

Signals were acquired using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). The currents obtained in the voltage-clamp mode were low-pass-filtered at 3 kHz and digitized at 333 kHz with an A/D converter (Digidata 1322A, Molecular Devices). The data were stored and analyzed with a personal computer using the pCLAMP 9.2 software (Molecular Devices). Spontaneous EPSCs and IPSCs (sEPSCs and sIPSCs, respectively) were detected and analyzed using Mini Analysis Program version 6.0.3 (Synaptosoft, Decatur, GA); detection criteria for sEPSCs or sIPSCs included a 5-pA event threshold, their fast rise time and a decay curve that approximated to an exponential decay (Fujita et al. 2009). sEPSCs or sIPSCs during a period of 3 min varied in frequency and amplitude with time around their average with a deviation of 5%; owing to this variation, when they changed >5% following superfusion of a drug, the effect of this drug on the spontaneous transmission was considered to be effective.

To examine what kind of channels are involved in oxytocin currents, a voltage step (duration: 200 ms) from the Vh of − 70 mV to potentials ranging from −150 to +40 mV was given to SG neurons in the absence and presence of the oxytocin currents (see Luo et al. 2001).

Application of drugs. Drugs were applied by perfusing a solution containing the drugs of a known concentration, without an alteration in the perfusion rate and temperature. The solution in the recording chamber, with a volume of 0.5 ml, was completely replaced within 15 s. The drugs used were oxytocin, strychnine nitrate, norepinephrine, serotonin creatine sulfate, d(+)-glucosamine HCl, cherylethry chloride, U-73122 hydrate, N2, O2, O2, dibutyryladenosine 3',5'-cyclic monophosphate (db-cAMP) sodium salt, 2-aminoethoxydiphenyl borate (2-APB), dantrolene sodium, (−)-nicotine, carbamoylcholine (Sigma-Aldrich, St. Louis, MO), [Thr4,Gly6]oxytocin (TGGT), [d(CH3)4, Tyr(Me)2,Thr,Om3,des-Gly-NH2]vasotocin (dVTG), [Arg4,vasopressin trifluoroacetate salt (AVP), [d(CH3)4,Tyr(Me)2,Arg2]vasopressin (TMA; Bachem AG, Bubendorf, Switzerland), bicuculline methiodide, tetrodotoxin (TTX), adenosine (Wako, Osaka, Japan) and 6-cyano-7-nitroquinoline-2,3-dione (CNOX; Tocris Cookson, Bristol, UK). These drugs (except for CNOX, U-73122, 2-APB and dantrolene where dimethyl sulfoxide was used as solvent) were first dissolved in distilled water at 1,000 times the concentration to be used and then stored at −20°C. The stock solution was diluted to the desired concentration in Krebs solution immediately before use. The toxicity of nominally Ca2+-free, high-Mg2+ (5 mM), high-K+ (10 mM) or high-Ca2+ (10 mM) Krebs solution was adjusted by lowering the Na+ concentration of the Krebs solution. Low Na+ (26.2 mM) solution was made by substituting glucosamine HCl for NaCl. Since oxytocin (1, 10 μM) or an oxytocin-receptor agonist TOTG (1 μM) was effective in modulating synaptic transmission in superficial dorsal horn neurons of rodent spinal cord slices (Breton et al. 2008, 2009; Robinson et al. 2002), the concentration of oxytocin or TOTG used in the present study was 0.5 μM, except for experiments examining concentration dependencies for oxytocin actions.

Statistical analysis. Numerical data are presented as the mean ± S.E. Statistical significance was determined as P < 0.05 using paired or unpaired Student’s t-test. In all cases, n refers to the number of neurons studied.

RESULTS

Whole-cell recordings were obtained from 425 SG neurons. Stable recordings could be obtained from slices maintained in vitro for more than 12 h, and recordings could be made from single SG neurons for up to 4 h. All SG neurons
tested had resting membrane potentials that were more negative than \(-55\) mV (when measured in a current-clamp mode). In 67% of the neurons examined \((n = 204)\), oxytocin \((0.5 \mu M)\) superfused for 3 min produced an inward current at the \(V_H\) of \(-70\) mV, as seen in the upper trace of Fig. 1A, when examined by using patch-pipette solution containing K-gluconate. The oxytocin-induced inward current had the peak amplitude of \(12.6 \pm 0.5\) pA \((n = 145)\) at \(-70\) mV. All of the remaining neurons, except for three neurons \((1\%)\), where outward currents having the peak amplitude of \(17.5 \pm 6.3\) pA \((n = 3)\) were produced (not shown), did not change holding currents.

**Effect of oxytocin on glutamatergic excitatory transmission in SG neurons.** Spontaneous excitatory transmission was not affected by oxytocin \((0.5 \mu M)\) in all neurons examined (for example, see the lower traces of Fig. 1A). When quantitatively examined in many neurons, the frequency and amplitude of sEPSC around 1.5 min after the beginning of oxytocin superfusion were, respectively, \(100 \pm 1\% (P > 0.05)\) and \(99 \pm 1\% (P > 0.05)\) of those \((9.5 \pm 0.5\) Hz and \(9.1 \pm 0.2\) pA; \(n = 174)\) before its superfusion.

Stimulating the dorsal root with strength of more than 20 \(\mu A\) (sufficient to recruit A\(\delta\) fibers) elicited monosynaptic glutamatergic EPSCs in some neurons. CV values estimated from the latency of the monosynaptic EPSC averaged to be \(4.5 \pm 0.4\) m/s \((3.7–5.3\) m/s; \(n = 4)\). This was within the range of those of A\(\delta\) fibers, as reported previously \((\text{Ataka et al. 2000; Yue et al. 2011})\). Monosynaptic A\(\delta\)-fiber EPSCs evoked at 0.1 Hz had a mean amplitude of \(65 \pm 21\) pA \((n = 4)\) at \(-70\) mV. On the other hand, stimuli with a strength larger than 250 \(\mu A\) (enough to activate C fibers) evoked monosynaptic glutamatergic EPSCs in some neurons. Primary-afferent fibers involved in the monosynaptic EPSCs had an average CV of \(0.51 \pm 0.02\) m/s \((0.46–0.57\) m/s; \(n = 5)\), with values comparable to those of C fibers \((\text{Ataka et al. 2000; Yue et al. 2011})\). Monosynaptic C-fiber EPSCs evoked at 0.1 Hz had a mean amplitude of \(132 \pm 22\) pA \((n = 5)\) at \(-70\) mV. Some SG neurons exhibited both monosynaptic A\(\delta\)-fiber and C-fiber EPSCs. As seen in Fig. 1B, each of the monosynaptic A\(\delta\)-fiber and C-fiber EPSCs were unaffected in peak amplitude by oxytocin \((0.5 \mu M)\). Similar results were seen in all neurons examined \((n = 4\) and 5 for A\(\delta\)-fiber and C-fiber EPSCs, respectively; Fig. 1C).

**Inward currents produced by oxytocin in SG neurons.** The oxytocin current decayed following its peak in the presence of oxytocin \((0.5 \mu M)\) superfused for 3 min, although this decay was variable in rate among neurons (not shown). This variability would be due to a difference among the neurons in the rate of oxytocin-receptor desensitization \((\text{Gimpl and Fahrenholz 2001})\) or the degradation of oxytocin by peptidases \((\text{Tsujimoto and Hattori 2005})\). Therefore, this was not quantitatively analyzed. When applied once again at various intervals after washout, the peak amplitude of oxytocin current produced by its second application approached that of the first application with an increase in the interval of time. At 5-min interval, the peak inward current in the second application was much smaller in amplitude than that of the first application (not shown). At 5- to 30-min intervals the rising rate of the inward current in the second application was smaller than that in the first application (not shown). Figure 2A demonstrates the peak amplitudes of oxytocin currents at the first and second applications, which are plotted against time intervals between the two applications \((n = 4, 5\) and 3 in 5-, 10- and 60-min intervals, respectively). This result indicates a slow recovery from desensitization. A ratio of the second
to the first oxytocin peak current amplitude at a time interval of 20–30 min averaged to be 0.77 ± 0.04 (n = 10). It took more than 1 h to completely recover from desensitization (see Fig. 2A).

In the presence of a voltage-gated Na⁺-channel blocker TTX (0.5 μM), oxytocin (0.5 μM) produced an inward current (Fig. 2B1, right), the peak amplitude of which was somewhat smaller than that in the absence of TTX 30 min before its application in the same neuron (Fig. 2B1, left). When examined in several neurons (n = 6), a ratio of the second to the first peak amplitude was not significantly different from that obtained by the repeated application of oxytocin in the absence of TTX (see above; P > 0.05; Fig. 2C). Spontaneous excitatory transmission was not affected by TTX itself, as noted from Fig. 2B1, right. sEPSC frequency and amplitude values in the absence and presence of TTX were, respectively, 7.0 ± 1.7 Hz, 10.5 ± 0.7 pA and 6.9 ± 1.8 Hz, 10.4 ± 0.7 pA; they were significantly unchanged by TTX (P > 0.05). Superfusing a non-N-methyl-D-aspartate receptor antagonist CNQX (10 μM) blocked the occurrence of the sEPSCs, under the condition of which oxytocin (0.5 μM) still produced an inward current (Fig. 2B2, right). A ratio of oxytocin peak current amplitudes in the absence and presence of CNQX was not significantly different from that obtained from its repeated application in the absence of CNQX (n = 6; P > 0.05; Fig. 2C). Moreover, the oxytocin current persisted in a nominally Ca²⁺-free Krebs solution (Fig. 2B3, right), where sEPSC frequency was reduced by 15 ± 4% (n = 6; P < 0.05) from 8.9 ± 3.1 Hz to 7.7 ± 2.9 Hz without a change in sEPSC amplitude (9.5 ± 1.2 pA and 9.5 ± 0.9 pA; for a similar result see Yue et al. 2011). Since a fourfold increase in extracellular Ca²⁺ concentration (10 mM) resulted in sEPSC frequency increase (by 215 ± 87%, n = 4; not shown) 2 min after the onset of this Ca²⁺ increase, as reported in rat superficial dorsal horn neurons by Hori et al. (1992), spontaneous excitatory transmission in SG neurons depended on extracellular Ca²⁺ entry. As seen in Fig. 2C, Ca²⁺-free also did not affect the inward current produced by oxytocin (n = 6; P > 0.05). These results indicate that the inward current is due...
to a direct action of oxytocin. We, therefore, examined a concentration dependency for the inward current produced by oxytocin. Since the oxytocin current was slow in recovery from desensitization, this current at each concentration was obtained from different neurons > 1 h after its washout. Oxytocin-induced current increased in rising rate and peak amplitude with an increase in its concentration in a range of 0.05–5 μM (not shown). The Hill analysis indicated that a half-maximal effective concentration (EC₅₀) for the peak amplitude is 0.022 μM (Fig. 2D).

Next, we examined the pharmacological property of the oxytocin response. The oxytocin current was mimicked by TGOT (0.5 μM), as seen in Fig. 3A. In 85% of the neurons tested (n = 13), TGOT produced an inward current having the peak amplitude of 9.2 ± 1.6 pA (n = 11). An oxytocin (0.5 μM)-insensitive SG neuron did not respond to TGOT (0.5 μM; Fig. 3B); a similar result was obtained from three more neurons. As seen from Fig. 3C, the inward current produced by oxytocin (0.5 μM) disappeared in the presence of an oxytocin-receptor antagonist dVOT (1 μM). Figure 3D demonstrates each of the dVOT effects obtained from six neurons. These observations indicate an involvement of oxytocin receptors in the inward current. Consistent with the fact that oxytocin receptors are coupled to G proteins, when the patch-pipette solution contained GDPβS (1 mM), the oxytocin response was blocked 60 min after the whole-cell configuration, as seen from Fig. 3E. The GDPβS effects obtained from individual five neurons are given in Fig. 3F.

Fig. 3. The inward current produced by oxytocin (0.5 μM) is mediated by oxytocin receptors. A: chart recording of an oxytocin-receptor agonist [Thr⁴, Gly⁷]oxytocin-induced (TGOT; 0.5 μM) inward current. B: in a neuron where oxytocin had no effect on holding currents, TGOT applied at 30 min after the oxytocin application did not produce any inward currents. C: chart recordings showing changes in holding currents following oxytocin superfusion in the absence (left) and presence of an oxytocin-receptor antagonist [d(CH₂)₅,Tyr(Me)²,Thr⁴,Orn⁸,des-Gly-NH₂⁹]vasotocin (dVOT, 1 μM; right), where oxytocin was given in a time interval of 30 min. D: the peak amplitudes of oxytocin-induced inward currents in the absence and presence of dVOT. Data obtained from the same neuron are connected by a straight line. E: chart recordings showing changes in holding currents following oxytocin superfusion just after (left) and 60 min after the whole-cell configuration under the condition where the patch-pipette solution contained GDPβS (1 mM; right). F: the peak amplitudes of oxytocin-induced inward currents just after (0 min) and 60 min after the whole-cell configuration, where data obtained from the same neuron are connected by a straight line. Vm = −70 mV.
Since oxytocin receptors are known to trigger $G_q$, $G_s$ or $G_i$ protein-mediated cellular signaling cascades (Stoop 2012), we examined which of the cascades underlie the inward current produced by oxytocin (0.5 μM). Under pretreatment with U-73122 (10 μM; Kobrinsky et al. 2000), an inhibitor of phospholipase C (PLC) coupled to $G_q$ protein, for 4 min, oxytocin produced an inward current (Fig. 4A, right), the peak amplitude of which was much smaller than that in the absence of U-73122 30 min before its application in the same neuron (Fig. 4A, left). Hydrolysis of phosphatidylinositol 4,5-bisphosphate by PLC results in the generation of two second messengers, diacylglycerol, which activates protein kinase C (PKC), and inositol 1,4,5-triphosphate (IP$_3$), which releases Ca$^{2+}$ from intracellular Ca$^{2+}$ stores. Under pretreatment with an IP$_3$-induced Ca$^{2+}$-release (IICR) inhibitor 2-APB (200 μM; Maruyama et al. 1997) for 4 min, oxytocin produced a small inward current (Fig. 4B, right), the peak amplitude of which was much smaller than that in the absence of the drug 30 min before its application in the same neuron (Fig. 4B, left). Figure 4F summarizes oxytocin current amplitude in its second application in the presence of U-73122 ($n = 4$) or 2-APB ($n = 4$), relative to that in its first application in the absence of the drug.

Fig. 4. The oxytocin (0.5 μM) current is mediated by phospholipase C (PLC) and inositol 1,4,5-triphosphate-induced Ca$^{2+}$ release (IICR), but not by protein kinase C (PKC), Ca$^{2+}$-induced Ca$^{2+}$ release (CICR) and cAMP. A–E: chart recordings of inward currents produced by oxytocin in the absence (left) and presence of a PLC inhibitor U-73122 (10 μM; A), an IICR inhibitor 2-aminoethoxydiphenyl borate (2-APB, 200 μM; B), a PKC inhibitor chelerythrine (10 μM; C), a CICR inhibitor dantrolene (10 μM; D) or dibutyl cyclic AMP (d-c-AMP, 1 mM; E: right) in the same neuron, where this peptide was applied at a time interval of 30 min. F: the peak amplitude of oxytocin current in the second application at 30 min interval in U-73122-, 2-APB-, chelerythrine-, dantrolene- or dibutyl cyclic AMP-containing Krebs solution, relative to that in the first application in normal Krebs solution from the same neuron (17.1 ± 1.0 pA, $n = 4$; 12.4 ± 2.7 pA, $n = 4$; 15.3 ± 3.2 pA, $n = 8$; 13.0 ± 1.7 pA, $n = 4$; and 13.4 ± 1.2 pA, $n = 4$; respectively), which was compared with that (the leftmost side, calculated from Fig. 2A) obtained by its repeated application in normal Krebs solution. Values in parentheses denote the no. of neurons tested. In this and subsequent figures, $^*P < 0.05$. $V_{st} = −70$ mV.
These drugs significantly inhibited the oxytocin current. On the other hand, a PKC inhibitor chelerythrine (10 μM; Herbert et al. 1990) and a Ca\(^{2+}\)-induced Ca\(^{2+}\)-release inhibitor dantrolene (10 μM; Nohmi et al. 1991; Ohta et al. 1990) pretreated for 4 min did not affect the oxytocin activity (Fig. 4, C and D).

A membrane-permeable analog of cAMP (intracellular concentration of which is regulated through G\(_a\) and G\(_i\) proteins), db-cAMP (1 mM), itself did not change holding currents (Fig. 4E). In the presence of db-cAMP, oxytocin produced an inward current having a peak amplitude comparable to that of the first application (Fig. 4E). As summarized in Fig. 4F, chelerythrine (n = 8), dantrolene (n = 4) and db-cAMP (n = 4) did not significantly affect the oxytocin current when tested in 4–8 neurons.

We next examined what kinds of channels mediate the oxytocin (0.5 μM) current by evaluating membrane currents in response to voltage pulses having a duration of 200 ms, as shown in Fig. 5, A1 and A2, top. Relationships between the step voltage and steady current at the end of its pulse, which were obtained in the absence and presence of the oxytocin current, are given in Fig. 5, A1 and A2, bottom. In 35% of neurons exhibiting oxytocin activity (n = 17), a net of oxytocin current, estimated from a difference between the two currents, reversed at a potential hyperpolarized from resting membrane potentials, as seen in Fig. 5A1. The reversal potential averaged to be −107 ± 7 mV (n = 6). This reversal potential (−119 ± 7 mV), allowed for a liquid junction potential of 12 mV existing between Krebs and K\(^{+}\)-glutonate containing patch-pipette solutions (Fujita and Kumamoto 2006), was somewhat hyperpolarized more than the equilibrium potential (E\(_K\); −98 mV) for K\(^{+}\), as calculated from the Nernst equation using K\(^{+}\) concentrations (3.6 and 140 mM, respectively) of these solutions. The other neurons did not show such a reversal (n = 11; for example see Fig. 5A2). In the neurons exhibiting reversal, oxytocin current had the slope conductances of 0.23 ± 0.07 nS (n = 4) and 0.47 ± 0.06 nS (n = 4) in ranges of −150 to −90 mV and of −70 to −50 mV, respectively; the former was significantly smaller than the latter (P < 0.05), indicating a decrease in membrane conductance with hyperpolarization.

Current-voltage relations in a wide range including voltages more positive than −40 mV were examined in the presence of TTX (0.5 μM). In a neuron exhibiting the reversal potential, as shown in Fig. 5A1, a net oxytocin current became to be large in peak amplitude at voltages more positive than −40 mV (Fig. 5A3). The reversal potential averaged to be −124 ± 3 mV (n = 4). On the other hand, in another neuron exhibiting only inward oxytocin current at voltages more negative than −40 mV, as shown in Fig. 5A2, the inward current became to be small in amplitude with depolarization and then reversed around 0 mV (Fig. 5A4). On average, the reversal potential was −8 ± 6 mV (n = 4).

In a neuron having a peak outward current amplitude of 30 pA, produced by oxytocin, a relationship between voltage and current in the absence and presence of this peptide showed a reversal potential of −87 mV for oxytocin current (not shown), indicating an involvement of K\(^{+}\) channels.

We, moreover, examined how the oxytocin activity is affected by an increase in extracellular K\(^{+}\) concentration (from 3.6 to 10 mM; Fig. 5B) or a decrease (by 117 mM; Fig. 5C) in extracellular Na\(^{+}\) concentration. In the high-K\(^{+}\) and low-Na\(^{+}\) Krebs solutions, oxytocin produced very small inward currents having the peak amplitudes of 5.0 ± 0.5 pA (n = 4) and 1.3 ± 0.8 pA (n = 4), respectively. Here, there were oxytocin-induced inward currents having the peak amplitudes of 13.8 ± 1.4 pA (n = 4) and 12.5 ± 0.7 pA (n = 4), respectively, in normal Krebs solution. Since muscarine-sensitive K\(^{+}\) channels, which produced a membrane depolarization (Adams et al. 1982; Kuba and Koketsu 1976), seemed to be involved in the oxytocin activity, we examined how Ba\(^{2+}\) at 1 mM, a concentration enough to inhibit muscarine-sensitive K\(^{+}\) channels (Adams et al. 1982), affects the inward current produced by oxytocin (0.5 μM). Since Ba\(^{2+}\) itself produced an inward current (depolarization; see Luo et al. 2001), resulting in the production of action potentials, this experiment was performed in the presence of TTX (0.5 μM; see Fig. 4D). Under pretreatment with Ba\(^{2+}\) for 4 min, oxytocin produced a small inward current, compared with that in the absence of Ba\(^{2+}\) 30 min before its application in the same neuron. The peak amplitudes of oxytocin currents in the absence and presence of Ba\(^{2+}\) were 14.1 ± 1.9 pA (n = 5) and 5.8 ± 1.9 pA (n = 5), respectively, where Ba\(^{2+}\) current had the peak amplitude of 22.4 ± 3.0 pA (n = 5). A ratio of the second to the first peak amplitude (0.38 ± 0.08; n = 5) was significantly smaller than that obtained by the repeated application of oxytocin in the absence of Ba\(^{2+}\) (0.78 ± 0.05; n = 8; P < 0.05).

The inward current produced by oxytocin at −70 mV, i.e., membrane depolarization, increases the membrane excitability of SG neurons, a result different from those produced by endogenous analgesics such as norepinephrine (North and Yoshimura 1984; Sonohata et al. 2004), serotonin (Abe et al. 2009; Ito et al. 2000) and adenosine (Li and Perl 1994; Liu et al. 2004). Therefore, we examined how such analgesics affect holding currents in SG neurons where oxytocin (0.5 μM) produces an inward current. Norepinephrine (20 μM), serotonin (40 μM) or adenosine (1 mM) produced an outward current at −70 mV in the oxytocin-sensitive neurons (not shown). The oxytocin-sensitive neurons having the peak inward current amplitudes of 13.5 ± 1.9 pA (n = 5), 13.9 ± 2.1 pA (n = 4) and 14.0 ± 2.1 pA (n = 4) responded to norepinephrine, serotonin and adenosine, respectively; they had the peak outward current amplitudes of 58.2 ± 8.1 pA (n = 5), 50.4 ± 7.9 pA (n = 4) and 7.5 ± 1.7 pA (n = 4), respectively.

On the other hand, in oxytocin-sensitive SG neurons, a nicotinic acetylcholine (ACh) receptor agonist (−)-nicotine (100 μM) having an antinociceptive effect (Khan et al. 1998) produced an inward current (not shown), as reported previously (Takeda et al. 2003). The peak amplitudes of the inward currents produced by oxytocin and (−)-nicotine were 13.4 ± 3.0 pA (n = 4) and 8.9 ± 1.5 pA (n = 4), respectively. In three out of four neurons where oxytocin produced inward currents having the peak amplitudes of 17.5 ± 1.6 pA (n = 4), a muscarinic ACh receptor agonist carbachol (10 μM), which acted as an analgesic (Abram and O’Connor 1995), elicited inward currents having the peak amplitudes of 7.0 ± 0.9 pA (n = 3). Remaining one neuron produced an outward current having the peak amplitude of 9.0 pA (not shown). Consistent with these results, Baba et al. (1998) have reported that carbachol elicits either outward or inward currents in the majority of the adult rat SG neurons tested.

**Effect of oxytocin on inhibitory transmission in SG neurons.**

Many of endogenous substances such as norepinephrine, sero-
tonin and ACh acting as analgesics in the spinal dorsal horn 
enhance spontaneous inhibitory transmission in SG neurons 
(Abe et al. 2009; Baba et al. 1998, 2000; Fukushima et al. 
2009; Takeda et al. 2003; also see Liu et al. 2011). GABAergic 
and glycinergic sIPSCs could be encountered in SG neurons. 
Since GABAergic and glycinergic spontaneous transmissions 
were affected by a phospholipase A2 activator melittin in a 
manner different from each other (Liu et al. 2008, 2011) and 
thus oxytocin appeared to affect each of the transmissions in a 
different manner (Breton et al. 2008), we examined the effect 
of oxytocin on both of the inhibitory transmissions. 
As seen in Fig. 6A, oxytocin (0.5 μM) enhanced GABAergic 
spontaneous transmission, which was observed in the presence of 
a glycine-receptor antagonist, strychnine (1 μM). In the presence
of a GABA_A-receptor antagonist, bicuculline (10 μM), glyciner-
gic sIPSCs could be recorded. Like GABAergic transmission, glyciner-
gic one was enhanced by oxytocin (0.5 μM; Fig. 6B). Figure 6C demonstrates the frequencies and amplitudes of GABAergic and glycineric sIPSCs before and 2 min after the onset of oxytocin superfusion in neurons having changes of >5%, where the proportions of neurons showing the GABAe-
ergic sIPSC frequency and amplitude increases were 92% and 77%, respectively, and neuronal proportions showing glyciner-
gic sIPSC frequency and amplitude increases were 96% and 68%, respectively. GABAergic sIPSC frequency and amplitude were increased by 438 ± 81% (P < 0.05; n = 24) and 66 ± 9% (P < 0.05; n = 20), respectively; glycineric ones were by 578 ± 70% (P < 0.05; n = 27) and 35 ± 7% (P <
0.05; n = 19), respectively.

Like the depolarized effect of oxytocin, in each of GABAergic
(Fig. 7A) and glycinergic transmissions (Fig. 7B), the sIPSC frequency and amplitude increases were repeated at an interval of 30 min, although the second effect was a little smaller in extent than the first one. Figure 7, C1 and C2, demonstrates a comparison of the frequency and amplitude of GABAergic or glycineric sIPSC under the action of oxytocin, relative to those before its superfusion, between the first and second applications, which was obtained from many neurons (n = 8 and 6 for GABAergic and glycineric sIPSCs, respectively). The relative GABAergic sIPSC frequencies in the first and second applications averaged to be 4.0 ± 0.6 (n = 8) and 2.9 ± 0.6 (n = 8), respectively; the former was significantly larger than the latter (P < 0.05). The relative GABAergic sIPSC amplitude in the first application (1.7 ± 0.2; n = 8) was also significantly larger than that in the second application (1.2 ± 0.1; n = 8; P < 0.05). On the other hand, the relative glycineric sIPSC frequency in the first application (3.9 ± 0.7; n = 6) had a tendency to be larger that in the second application (2.5 ± 0.3; n = 6); the relative glycineric sIPSC amplitude in the first application (1.6 ± 0.1; n = 6) had a tendency to be also larger than that in the second application (1.4 ± 0.1; n = 6).

Since the inhibitory transmission enhancement produced by oxytocin was slow in recovery from desensitization, this oxy-
tocin effect at each concentration was obtained from different neurons >1 h after its washout. Figure 7, D1 and D2, demon-
strates concentration-response relationships for GABAergic and glycineric sIPSC frequency and amplitude values, respec-
tively, relative to those before oxytocin superfusion, in a range of 0.005 to 1 μM. Analyses based on the Hill equation showed that EC_{50} values for oxytocin in increasing GABAergic and glycineric sIPSC frequencies were 0.024 μM and 0.038 μM, respectively. It is noted from Figs. 6 and 7 that holding currents change in the presence of oxytocin in some neurons. This may have been due to a change in K^+ and/or Na^+ permeability as a result of oxytocin receptor activation. However, oxytocin (0.5 μM) did not change holding currents in the presence of bicuculline (10 μM) and strychnine (1 μM) in all neurons examined (n = 4; not shown). The oxytocin-induced change in holding currents will be possibly due to a summation of sIPSCs, as seen in the action of carbamoylcholine (Baba et al. 1998; Liu et al. 2011) or melittin (Liu et al. 2008) on sponta-
neous inhibitory transmission in SG neurons.

To know whether the sIPSC frequency and amplitude in-
creases produced by oxytocin are accompanied by an increase in neuronal activities, we next examined whether the oxytocin
effects are affected by TTX (0.5 μM). As seen from Fig. 8, A and B, oxytocin (0.5 μM) did not enhance GABAergic and glycineric spontaneous transmissions in the presence of TTX, when examined in the same neuron. Figure 8C1 demonstrates GABAergic sIPSC frequency and amplitude values before and around 2 min after the onset of oxytocin superfusion in Krebs solution without and with TTX (n = 4). A similar result for glycineric sIPSCs is given in Fig. 8C2 (n = 4). Spontaneous inhibitory transmission was not significantly affected by TTX itself, as noted from Fig. 8, A and B, right. GABAergic sIPSC frequency and amplitude in the absence and presence of TTX were, respectively, 3.9 ± 1.6 Hz (n = 5), 10.2 ± 1.9 pA (n = 5) and 3.6 ± 1.5 Hz (n = 5), 9.9 ± 1.9 pA (n = 5); glycineric ones in the absence and presence of TTX were, respectively, 4.1 ± 1.9 Hz (n = 5), 10.3 ± 1.1 pA (n = 5) and 3.9 ± 1.9 Hz (n = 5), 10.0 ± 1.1 pA (n = 5). These results indicate that the effects of oxytocin on inhibitory transmissions are due to an increase in neuronal activities, possibly as a result of a depo-
larizing effect of oxytocin on inhibitory SG neurons.

We further examined the pharmacological property of the faciliatory effects of oxytocin on GABAergic and glycineric transmis-
sions. The inhibitory transmission enhancements produ-
ced by oxytocin were mimicked by TGOT (0.5 μM), as seen in
Fig. 9, A and B. In all neurons tested (n = 5–6), TGOT increased GABAergic and glycineric sIPSC frequency; their frequency values in the absence and presence of TGOT were given in Fig. 9C, left. TGOT also increased GABAergic and glycineric sIPSC amplitudes in almost all neurons examined (n = 4–5; Fig. 9C, right). These TGOT effects had a tendency to be smaller than those of oxytocin (Fig. 6C) in sIPSC frequency and amplitude increase. The relative GABAergic sIPSC frequencies in the oxytocin and TGOT effects averaged to be 5.38 ± 0.87 (n = 24) and 1.92 ± 0.28 (n = 6), respectively; the former was significantly larger than the latter (P < 0.05). The relative glycineric sIPSC frequencies in the oxytocin and TGOT effects were 5.96 ± 0.69 (n = 27) and 3.08 ± 0.38 (n = 5), respectively. Moreover, the relative GABAergic sIPSC amplitudes in the oxytocin and TGOT

Fig. 5. The inward current produced by oxytocin (0.5 μM) is mediated by changes in the membrane permeability of K^+ and/or Na^+. A1–A4: the effect of oxytocin on current-voltage relation. Recordings of oxytocin-induced inward currents, where voltage pulses were given in the absence and presence of this peptide (top in each of A1 and A2), are shown. Here, membrane currents in response to voltage pulses (duration: 200 ms) from the V_m of −70 mV in the absence (bottom left) and presence of oxytocin (bottom right) are shown. In the bottom of A1 and A2, the amplitudes of membrane currents measured at the end of voltage pulses are plotted against voltages, where they are obtained in the absence (●) and presence of oxytocin (▲). Current-voltage relationships for a net of oxytocin current, estimated from a difference between the current responses in the absence and presence of this peptide, are shown by solid circles (●). Note that the net current in A1 reverses, while that in A2 does not. A3 and A4 show current-voltage relations for net oxytocin current in a wide voltage range in the presence of TTX (0.5 μM). It is noted that the inward current becomes large in amplitude with polarization in A3, and that the current-voltage relation in A4 reverses at a potential close to 0 mV. B and C: recordings of oxytocin-induced inward currents in normal (left) and high K^+ (10 mM) or low Na^+ (decreased by 117 mM) Krebs solution (right). D: recordings of oxytocin currents in the absence and presence of Ba^2+ (1 mM), where TTX (0.5 μM) was added to inhibit the production of action potentials. In B–D, oxytocin was given in a time interval of 30 min. V_m = −70 mV.

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effects were 1.66 ± 0.09 (n = 20) and 1.28 ± 0.17 (n = 4), respectively; the relative glycinergic sIPSC amplitudes in the oxytocin and TGOT effects were 1.55 ± 0.06 (n = 19) and 1.27 ± 0.03 (n = 5), respectively.

The GABAergic and glycinergic transmission enhancements produced by oxytocin (0.5 µM) disappeared by a pretreatment for 4 min with dVOT (1 µM), as seen in Fig. 9, D and E. Figure 9F1 demonstrates GABAergic sIPSC frequency and amplitude values before and around 2 min after the onset of oxytocin superfusion in Krebs solution without and with dVOT, examined in the same neuron (n = 5). A similar result for glycinergic sIPSCs (n = 4) is given in Fig. 9F2. These observations indicate an involvement of oxytocin receptors in increasing (GABAergic and glycinergic) sIPSC frequency and amplitude.

The oxytocin-induced inward currents are not mediated by vasopressin V1A receptors. Oxytocin can bind to and activate vasopressin receptors, albeit with lower affinity and efficacy (Chini et al. 2008). Schorscher-Petcu et al. (2010) have reported that antinociception produced by the systemic administration of oxytocin is mediated by vasopressin V1A receptors. We therefore examined how the inward current produced by oxytocin (0.5 µM) in SG neurons is affected by a vasopressin V1A receptor antagonist TMA (1 µM). As seen from Fig. 10A, TMA inhibited the oxytocin activity, indicating an involvement of vasopressin V1A receptors. The peak amplitudes of oxytocin currents in the absence of TMA and 30 min later in the presence of TMA were 14.3 ± 3.1 pA (n = 4) and 6.9 ± 0.6 pA (n = 4), respectively. A ratio of the former to the latter value (0.52 ± 0.06; n = 4) was significantly smaller than that of the second to the first oxytocin current amplitude in the absence of TMA at a time interval of 30 min (0.78 ± 0.05; n = 8; P < 0.05).

If the oxytocin response in SG neurons is mediated by vasopressin V1A receptors, it is expected that a vasopressin-receptor agonist AVP produces an inward current as seen for oxytocin, and thus that there is a correlation in peak amplitude between the two inward currents. Therefore, we next examined both oxytocin and AVP (each 0.5 µM) responses in the same SG neuron (n = 11). Some of neurons sensitive to oxytocin exhibited an AVP response (Fig. 10B1), while many of the neurons did not respond to AVP (Fig. 10B2). Figure 10C demonstrates the peak amplitude of the AVP current, which is plotted against that of the oxytocin current. There was no correlation between the two current amplitudes (correlation coefficient: 0.21).

**DISCUSSION**

All SG neurons examined exhibited glutamatergic sEPSCs, GABAergic and glycinergic sIPSCs. These sEPSCs and sIPSCs were not significantly affected in both frequency and amplitude by Na+ channel blocker TTX, as reported previously (for example see Liu et al. 2008; Luo et al. 2002; Yue et al. 2011), possibly owing to deafferentiation in the slices used. This indicated that all of them occurred without the propagation of spikes from cell soma, whose neuron was presynaptic to SG neurons, to the terminals, resulting in spontaneous releases. The present study demonstrated that oxytocin at 0.5 µM (maximally-activating concentration) produces an inward current at ~70 mV (membrane depolarization) by activating oxytocin receptors in 67% of the adult rat SG neurons examined, albeit this amplitude is variable among the neurons. This variability in proportion and amplitude will be possibly due to the fact that the SG is composed of a heterogeneous cell group (Grudt and Perl 2002). Only 1% of the neurons exhibited an outward current. On the other hand, spontaneous, monosynaptically-evoked primary-afferent Aδ-fiber and C-fiber glutamatergic transmissions were not affected by oxytocin in all neurons tested.

The inward current produced by oxytocin was slow in recovery from desensitization; it took 20–30 min after its washout for recovery to about 80% of control. A slow recovery...
from desensitization has been reported for an increase in intracellular Ca\(^{2+}\) concentration produced by oxytocin-receptor activation in the gonadotrope-derived αT3-1 cell line; the second application of oxytocin had no effect around 3 min after its first application (Evans et al. 1997). Although the concentration (0.5 μM) of oxytocin used in our desensitization experiment was larger than its EC\(_{50}\) value (0.022 μM), this did not appear to be related to such a slow recovery from desensitization, because Evans et al. (1997) used a low concentration such as 0.1 μM of oxytocin in the above-mentioned experiment. Such a slow recovery may be due to the fact that oxytocin-receptor desensitization is caused by an internalization of the receptor (Gimpl and Fahrenholz 2001). As different from our result, oxytocin responses in young rat vagal motoneurons appeared to exhibit a fast, not slow, recovery from desensitization (Alberi et al. 1997). The inward current in our study was resistant to TTX and non-N-methyl-D-aspartate receptor antagonist CNQX, and persisted in Ca\(^{2+}\)-free solution, suggesting a direct action of oxytocin.

The oxytocin current in SG neurons exhibited two types of current-voltage relation: one reversed at potentials (−119 mV) hyperpolarized more than \(E_K\) (−98 mV), and the other reversed near 0 mV. These results indicate an involvement of changes in membrane permeabilities to K\(^+\) and/or other cations in the oxytocin current. This may be the reason why the former reversal potential is negative more than \(E_K\). It is unlikely that the oxytocin current is mediated by Ca\(^{2+}\) influx, because the oxytocin current is unaffected by removing extracellular Ca\(^{2+}\). In high-K\(^+\) or low-Na\(^+\) Krebs solution, oxytocin produced a small inward current, suggesting that the oxytocin activity is mediated by a change in membrane permeabilities to K\(^+\) and/or Na\(^+\). The inward current is expected to be produced by the closure of K\(^+\) channels (that open at −70 mV, as seen for muscarine-sensitive K\(^+\) channels; see Nicholls et al. 2001) and/or the opening of Na\(^+\) channels (that close at −70 mV). If K\(^+\)-channel closing occurs, an inward or outward current would flow through membrane at potentials more positive or more negative than \(E_K\), respectively, as shown in
In the neurons exhibiting a reversal at potentials hyperpolarized more than $E_K$, the slope conductance at hyperpolarizing potentials was smaller than that at depolarizing potentials, suggesting a closure of channels involved in the production of oxytocin current. If Na$^{+}$/H$^{+}$-channel opening occurs, an inward current would flow through membrane at negative potentials, as shown in Fig. 5A2 (see Kuba and Koketsu 1976). This involvement of Na$^{+}$/H$^{+}$-channel opening is supported by the observation that the inward current became to be outward at near 0 mV (Fig. 5A4). Breton et al. (2009) have reported that oxytocin closes A-type and delayed-rectifier K$^{+}$/H$^{+}$-channels in young rat SG neurons. Since these K$^{+}$/H$^{+}$-channels do not open at $V_m$ = 70 mV, another types of K$^{+}$/H$^{+}$ channel will be closed by oxytocin. Oxytocin may close muscarine-sensitive K$^{+}$/H$^{+}$ channels, because the oxytocin current is sensitive to Ba$^{2+}$ (Adams et al. 1982) and carbamoylcholine produces inward currents in SG neurons. Although Raggenbass and Dreifuss (1992) have reported that oxytocin generates a sustained Na$^{+}$-dependent current, which is insensitive to TTX, in young rat vagal motoneurons, it is unlikely that this Na$^{+}$ current is involved in the oxytocin current in our study. The sustained Na$^{+}$ current in motoneurons became to be bigger at voltages more positive than $-40$ mV, but this was not observed in the present study. It remains to be examined what types of K$^{+}$ and Na$^{+}$ channels in adult rat SG neurons are affected by oxytocin.

The depolarizing effect of oxytocin in the present study had the EC$_{50}$ value of 0.022 μM, a value similar to those (0.0266–0.0275 μM) in inhibiting or activating inwardly-rectifying K$^{+}$ currents in immortalizing gonadotropin-releasing hormone-positive GN11 cell lines (Gravati et al. 2010). On the other hand, the SG EC$_{50}$ value was smaller than one (0.1 μM) for oxytocin in producing inward currents in young rat vagal motoneurons (Raggenbass and Dreifuss 1992), while being larger than one (0.005 μM) in increasing firing rate in neonatal rat vagal motoneurons (Tribollet et al. 1989).

The oxytocin activity in our study was mimicked by oxytocin-receptor agonist TGOT and inhibited by oxytocin-receptor antagonist dVOT, indicating the activation of oxytocin receptors. This result is consistent with the presence of oxytocin receptors in the adult rodent spinal superficial dorsal horn (Moreno-López et al. 2013; Tribollet et al. 1997; Wrobel et al. 2011). Some of the SG neurons responded to vasopressin-receptor agonist AVP, indicating the presence of not only oxytocin but also vasopressin receptors in the SG. Consistent with the ability of
oxytocin to activate vasopressin receptors (Chini et al. 2008), the oxytocin current was inhibited by vasopressin V1A-receptor antagonist TMA. There was, however, no correlation in peak amplitude between currents produced by oxytocin and AVP, indicating that the oxytocin response was not mediated by vasopressin V1A receptors. As noted from Fig. 10C, a population of neurons responsive to TMA with a change of >5% in sIPSC frequency and amplitude to that of all neurons examined. Here, given are data obtained from neurons exhibiting changes of >5%. D and E: chart recordings of GABAergic (D) and glycineic sIPSCs (E) in the absence and presence of oxytocin in Krebs solution without (left) and with dVOT (1 μM; right). In each of D and E, the right recording was obtained 30 min after the left recording from the same neuron. F1 and F2: averages of the frequency and amplitude of GABAergic (F1; n = 5) or glycineic sIPSCs (F2; n = 4) just before (Control) and around 2 min after the beginning of oxytocin superfusion (Oxytocin) in the absence (–) and presence (+) of dVOT in the same neuron. 

**Fig.9.** The inhibitory transmission enhancement produced by oxytocin (0.5 μM) is mediated by oxytocin receptors. A and B: chart recordings of GABAergic (A) and glycineic sIPSCs (B) in the absence and presence of TGOT (0.5 μM). C: averages of the frequency and amplitude of GABAergic and glycineic sIPSCs just before (Control) and around 2 min after the beginning of TGOT superfusion (TGOT). Value in parentheses denotes a ratio of the no. of the neurons responsive to TGOT with a change of >5% in sIPSC frequency and amplitude to that of all neurons examined. Here, given are data obtained from neurons exhibiting changes of >5%. D and E: chart recordings of GABAergic (D) and glycineic sIPSCs (E) in the absence and presence of oxytocin in Krebs solution without (left) and with dVOT (1 μM; right). In each of D and E, the right recording was obtained 30 min after the left recording from the same neuron. F1 and F2: averages of the frequency and amplitude of GABAergic (F1; n = 5) or glycineic sIPSCs (F2; n = 4) just before (Control) and around 2 min after the beginning of oxytocin superfusion (Oxytocin) in the absence (–) and presence (+) of dVOT in the same neuron. 

1003EFFECT OF OXYTOCIN ON SYNAPTIC TRANSMISSION

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Because this outward current reverses at near-dependent K channels. The straight line drawn through the data points is the least squares regression line (correlation coefficient: 0.21). There was no correlation between the peak amplitudes of currents produced by AVP and oxytocin in the same neuron. Note that oxytocin produced an inward current in a neuron sensitive (B1) or insensitive to AVP (B2). C: the peak amplitude of AVP current, plotted against that of oxytocin current in the same neuron. The straight line drawn through the data points is the least squares regression line (correlation coefficient: 0.21). There was no correlation between the peak amplitudes of currents produced by AVP and oxytocin in the same neuron. V_H = -70 mV.

Consistent with the fact that oxytocin receptors are coupled to G proteins, intracellular GDPβS inhibited the inward current produced by oxytocin. The oxytocin current was inhibited by PLC inhibitor U-73122 or IICR inhibitor 2-APB, indicating PLC activation, resulting in the production of IP3 through Gq protein, which in turn activates IICR mechanisms. Che et al. (2012) have reported a U-73122 (10 μM)- and 2-APB (100 μM)-sensitive oxytocin response in cultured rat duodenum myenteric intrinsic primary-afferent neurons, although this is a membrane hyperpolarization due to the opening of Ca2+-dependent K+ channels. The oxytocin-induced outward current observed in the present study (see above) may have been due to the activation of Ca2+-dependent K+ channels as a result of an increase in intracellular Ca2+ concentration due to IICR, because this outward current reverses at near E_K.

Although oxytocin responses in our study were not completely suppressed by U-73122 or 2-APB, as reported by Che et al. (2012), this may be explained by the facts that U-73122 has an effect from the intracellular side with a slow time course (see Gravati et al. 2010), and that 2-APB at high concentrations causes a gradual release of Ca2+ from intracellular stores, an effect opposed to IICR inhibition (Maruyama et al. 1997). PKC activation did not appear to be involved in the oxytocin current, because of no effect of PKC inhibitor chelerythrine. Moreover, db-cAMP itself did not change holding currents and did not affect oxytocin currents, indicating no involvement of Gs and Gi proteins. It remains to be examined how intracellular Ca2+ increase by IICR mechanisms alters membrane permeabilities to K+ and/or Na+.

2-APB has been shown to block not only IP3 receptors on intracellular Ca2+ stores, but also transient receptor potential canonical (TRPC) 3 and 6 channels on plasma membranes (Hu et al. 2004; Trebak et al. 2002). The TRPC channels may have been activated by diacylglycerol produced as a result of oxytocin receptor and subsequent PLC activation (Vazquez et al. 2004), leading to inward currents in SG neurons. This possibility, however, appears to be unlikely, because oxytocin currents persist in Ca2+-free solutions (see Fig. 2B3), in spite of the fact that TRPC 3 and 6 channels have a high Ca2+ permeability (Vazquez et al. 2004). Moreover, although TRPC channel currents have a reversal potential close to 0 mV (Vazquez et al. 2004), this is the case in some but not all of oxytocin currents in our study, as seen from Fig. 5A. A possibility cannot be ruled out that TRPC channel activation is partly involved in the oxytocin response in SG neurons.

Unlike excitatory transmission, both GABAergic and glycinergeic spontaneous inhibitory transmissions were facilitated by oxytocin in adult rat SG neurons. This oxytocin action was mimicked by TGOT and disappeared in the presence of dVOT, indicating an involvement of oxytocin receptors. This facilitatory action was attributed to an increase in neuronal excitability as a result of inward current (membrane depolarization) produced by oxytocin, because oxytocin did not facilitate the inhibitory transmissions in the presence of TTX. Consistent with this idea, GABAergic and glycinergeic spontaneous inhibitory transmission frequency increases produced by oxytocin had EC50 values (0.024 and 0.038 μM, respectively) almost comparable to that (0.022 μM) of inward current. Moreover, the inhibitory transmission en-
hancements produced by oxytocin were slow in recovery from desensitization, as seen for its depolarizing action. The abilities of TGOT to enhance inhibitory transmissions and to produce inward currents were smaller than oxytocin’s ones, as seen for their presynaptic effects in neonatal rat spinal dorsal horn neurons (Jo et al. 1998). This result was different from the peripheral effects of TGOT and oxytocin, such as milk ejection and uterine contraction (Elands et al. 1988).

The present study found out for the first time that oxytocin produces a membrane depolarization, resulting in the enhancement of spontaneous GABAergic and glycinergic inhibitory transmissions in many of the adult rat SG neurons tested. This result is not accompanied by a change in spontaneous and primary-afferent evoked glutamatergic excitatory transmissions. Since we examine the effects of various drugs on oxytocin responses that partially recover from desensitization, a possibility cannot be ruled out that our results about the drug effects are partly affected by existing desensitization of oxytocin receptors that may be lacking in regular binding activity and active downstream signaling.

Our results were different from that reported by Breton et al. (2008) that TGOT increased sEPSC frequency in young rat spinal superficial dorsal horn neurons, and also from that by Robinson et al. (2002) that oxytocin inhibited primary-afferent excitatory transmission evoked in adult mouse spinal superficial dorsal horn neurons by stimulating the dorsal root entry zone without a change in resting membrane potentials. Moreover, Jo et al. (1998) have reported that oxytocin increases sEPSC frequency and electrically-evoked EPSC amplitude in a culture of spinal superficial dorsal horn neurons from neonate rats. TGOT produced a transient membrane depolarization in only 10% of young rat SG neurons tested (Breton et al. 2008). With respect to inhibitory transmissions, TGOT facilitated spontaneous GABAergic but not glycinergic transmission; this facilitatory action was attributed to an increase in the spontaneous release of L-glutamate onto GABAergic inhibitory neurons (Breton et al. 2008). Oxytocin actions in the spinal dorsal horn may alter with development and between rats and mice. Liu et al. (2003) have demonstrated that oxytocin-binding sites in the spinal superficial dorsal horn are much smaller in number in 9-wk than 1-wk-old rats (for a similar result, see Uhl-Bronner et al. 2005).

According to immunocytochemical studies, there are glutamate- and GABA- (at most 46% of the overall neuronal population) and/or glycine-containing (14%) neurons in the SG (Ribeiro-da-Silva and De Koninck 2009). Our results expect that oxytocin receptors are expressed in GABAergic, glycinergic but not glutamatergic neurons in the SG. However, Breton et al. (2008) have reported in the young rat SG that the electrical stimulation of hypothalamic paraventricular nucleus reveals c-Fos (a marker of neuronal activity) positive nuclei that exhibit a low glutamic acid-decarboxylase (GABA-synthesizing enzyme)-immunoreactivity, i.e., GABAergic neurons are hardly activated by the stimulation which is expected to release oxytocin. There are no cells which are doubly labeled for oxytocin receptors of human origin and a rabbit anti-GABA in adult rat laminae I-II (Moreno-López et al. 2013). Considering our observation that 67% of the neurons tested (which are located at the center of SG) are sensitive to oxytocin, more studies will be necessary to examine phenotypes of neurons expressing oxytocin receptors in the adult rat SG.

Physiological significance of the effects of oxytocin on synaptic transmissions in adult rat SG neurons. Hobo et al. (2012) have reported that oxytocin inhibits membrane depolarization-induced increase in intracellular Ca2+ concentrations in adult rat capsaicin-sensitive DRG neurons. Based on this observation, they suggested that a part of the antinociceptive effect of intrathecally-applied oxytocin may be due to a reduction of the release of L-glutamate to spinal superficial dorsal horn neurons from primary-afferent C-fiber terminals. This is, however, unlikely, because oxytocin does not change primary-afferent monosynaptic C-fiber EPSC amplitudes in adult rat SG neurons. Although Schorscher-Petcu et al. (2010) suggested that systemically-administered oxytocin-induced analgesia may be mediated by vasopressin V1A receptors in mouse DRG neurons, this action did not appear to be due to its action on the central terminals of primary-afferent neurons, because monosynaptic Aδ-fiber and C-fiber EPSC amplitudes in SG neurons were not affected by oxytocin.

The depolarizing effect of oxytocin, revealed in the present study, was different from the actions of endogenous analgesics, such as serotonin (Abe et al. 2009; Ito et al. 2000), adenosine (Li and Perl 1994; Liu et al. 2004) or norepinephrine (North and Yoshimura 1984; Sonohata et al. 2004), which produced an outward current (membrane hyperpolarization) in SG neurons sensitive to oxytocin with a membrane depolarization. Other endogenous analgesics including nociceptin (Lai et al. 1997; Luo et al. 2001), somatostatin (Jiang et al. 2003; Nakatsuka et al. 2008), opioids (Fujita and Kumamoto 2006; Wu et al. 1999; Yoshimura and North 1983), dopamin (Tamae et al. 2005; Taniguchi et al. 2011) and galanin (Alier et al. 2008; Yue et al. 2011) also produced a membrane hyperpolarization. The antinociceptive effect of oxytocin would be produced by its depolarizing effect, resulting in the enhancements of spontaneous GABAergic and glycinergic inhibitory transmissions, which in turn lead to an inhibition of the membrane excitability of SG neurons. This idea is supported by the observation that GABA_A-receptor antagonist bicuculline blocked antinociceptive responses produced by the electrical stimulation of hypothalamic paraventricular nucleus or oxytocin application (Condés-Lara et al. 2009a). An inhibitory transmission enhancement similar to that of oxytocin is produced by endogenous analgesics, such as norepinephrine and serotonin, although they also produce a membrane hyperpolarization and L-glutamate release reduction in SG neurons. The antinociceptive action of oxytocin appeared to be similar to those of nicotinic and muscarinic receptor activations by ACh (Abram and O’Connor 1995; Abram and Winnie 1995; Khan et al. 1998, 2001), because their ACh receptor activations enhanced spontaneous inhibitory transmissions together with a membrane depolarization (nicotine; Takeda et al. 2003) and a membrane depolarization or hyperpolarization (carbamoylcholine; Baba et al. 1998; Liu et al. 2011) in SG neurons.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.-Y.J., T.F., and E.K. conception and design of research; C.-Y.J. performed experiments; C.-Y.J. and T.F. analyzed data; C.-Y.J., T.F., and E.K. interpreted results of experiments; C.-Y.J., T.F., and E.K. approved final version of manuscript; T.F. prepared figures; E.K. drafted manuscript.

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