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Actions of a novel water-soluble benzodiazepine-receptor agonist JM-1232(−) on synaptic transmission in adult rat spinal substantia gelatinosa neurons

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ABSTRACT

Although the intrathecal administration of JM-1232(−) reportedly produces antinociception, this action has not yet been examined at the cellular level. We examined the action of JM-1232(−) on synaptic transmission in spinal substantia gelatinosa (SG) neurons which play an important role in regulating nociceptive transmission from the periphery. The whole-cell patch-clamp technique was applied to the SG neurons of adult rat spinal cord slices. Bath-applied JM-1232(−) prolonged the decay phase of $GABA_A$-receptor mediated spontaneous inhibitory postsynaptic current (sIPSC) and increased its frequency without a change in amplitude. The former but not latter action was sensitive to a benzodiazepine-receptor antagonist flumazenil. JM-1232(−) also increased glycinergic sIPSC frequency with no change in amplitude and decay phase. On the other hand, glutamatergic spontaneous excitatory transmission was unaffected by JM-1232(−). These results indicate that JM-1232(−) enhances inhibitory transmission by (1) prolonging the decay phase of GABAergic sIPSC through benzodiazepine-receptor activation and by (2) increasing the spontaneous release of GABA and glycine from nerve terminals without its activation. This enhancement could contribute to at least a part of the antinociceptive effect of intrathecally-administered JM-1232(−).

1. Introduction

An isoindoline derivative, JM-1232(−) {((−)-3-[2-(4-methyl-1-piperazinyl) -2-oxoethyl]-2-phenyl-3,5,6,7-tetrahydrocyclopenta[*f*]isoindole-1(2H)-one)}, is a novel water-soluble benzodiazepine-receptor agonist which is different in chemical structure from benzodiazepines (see Fig. 1A; for review see [28]). Although behavioral studies demonstrate that the intrathecal and intraperitoneal administration of JM-1232(−) produce antinociception in rodents [5,24], mechanisms for this action have not yet been examined at the cellular level. The substantia gelatinosa (SG; lamina II of Rexed) of the spinal dorsal horn is thought to play an important role in regulating nociceptive transmission to the CNS from periphery [30]. There is much evidence to support this idea. For instance, endogenous analgesics such as endomorphins and adenosine, which exhibit antinociception when administrated intrathecally, hyperpolarize membranes of SG neurons and reduce the release of L-glutamate onto SG neurons from nerve terminals, both of which result in reducing the excitability of the SG neurons ([11,19]; for review see [13]). The SG neurons receive not only glutamatergic excitatory but also (GABAergic and glycinergic) inhibitory transmission [30], the modulation of which may also play a role in regulating nociceptive transmission [7,23].

The SG has been found to express the highest density of $GABA_A$/benzodiazepine receptors in rats [3,6] and in humans [10,29]. When administrated intrathecally, a water-soluble imidazobenzodiazepine derivative, midazolam, produces antinociception in rats [2,15] and humans [14,27]. This action was inhibited by a $GABA_A$-receptor antagonist bicuculline and a benzodiazepine-receptor antagonist flumazenil [8,15,27], indicating an involvement of $GABA_A$/benzodiazepine receptors. This idea was supported by the observation that midazolam enhances a $GABA_A$-receptor response in a manner sensitive to flumazenil in rat SG neurons [18]. Benzodiazepines are known to have various actions including the inhibition of voltage-gated Ca^{2+} channel [4] or adenosine uptake [25] without benzodiazepine-receptor activation (for review see [26]). It is therefore possible that JM-1232(−) acts on sites other than $GABA_A$/benzodiazepine receptors, resulting in antinociception. In order to know cellular mechanisms for the antinociception produced by JM-1232(−), we examined its action on not only GABAergic but also glycinergic and glutamatergic transmissions in the SG neurons of adult rat spinal cord slices by using the patch-clamp technique.

2. Materials and methods

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

Adult rat spinal cord slice preparations were obtained in a manner similar to that described previously [12,20,31]. In brief, adult

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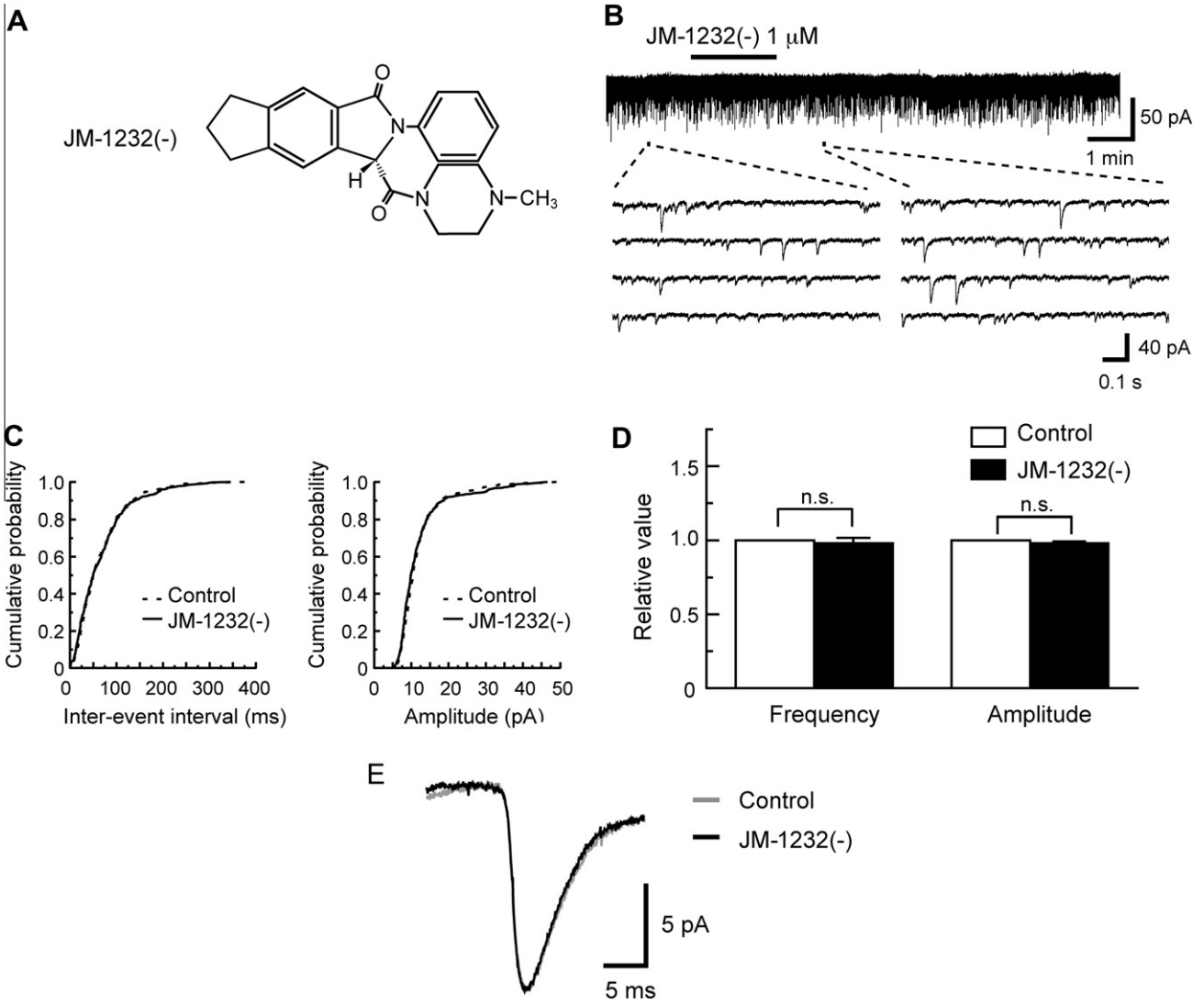

Fig. 1. JM-1232(−) (1 μM) does not affect spontaneous glutamatergic excitatory transmission in substantia gelatinosa (SG) neurons. (A) The chemical structure of JM-1232(−). (B) Chart recording of spontaneous excitatory postsynaptic currents (sEPSCs) in the absence and presence of JM-1232(−) superfused for 2 min. In this and subsequent figures, a bar shown above the recording indicates a period during which drugs are superfused; four traces, which are shown in an expanded scale in time, indicate synaptic currents recorded consecutively for a period indicated by a bar shown below the recording. (C) Cumulative histograms of the inter-event interval and amplitude of sEPSC in the control (dotted line; 453 sEPSC events) and around 3 min after the beginning of JM-1232(−) superfusion (continuous line; 445 sEPSC events), where the histograms were examined for 0.5 min. JM-1232(−) did not shift the distributions of the inter-event interval and amplitude of sEPSC ($P > 0.5$; Kolmogorov–Smirnov test). (D) The frequency and amplitude of sEPSC, relative to control (5.9 ± 1.2 Hz and 12.6 ± 0.9 pA; $n = 7$), measured around 3 min after the beginning of JM-1232(−) superfusion. In this and subsequent figures, vertical lines accompanied by bars indicate SEM; n.s.: not significant. (E) Averages of 279 sEPSC events in the control (gray line) and of 273 ones around 3 min after the beginning of JM-1232(−) superfusion (black line), which are superimposed. (B), (C) and (E) were obtained from the same neuron. Holding potential (V_H) = −70 mV.

male Sprague–Dawley rats (6–8 weeks old; 200–300 g) were anesthetized with urethane (1.5 g/kg body weight, i.p.) and a laminectomy was performed to extract a lumbosacral spinal cord enlargement (L1–S3). The spinal cord was carefully removed in a manner 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, $CaCl_2$ 1.2, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 25 and glucose 11) bubbled with 95% O_2 and 5% CO_2 (pH = 7.4). Rats were killed by exsanguination. A transverse slice (650–700 μm thick) without dorsal roots was cut using a microslicer (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% O_2 and 5% CO_2, and maintained at 36 ± 1 °C.

The SG was identified as a translucent band under a binocular microscope with light transmitted from below, as done previously [12,20,31]. 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 o.d.) and contained the following solution (in mM): K-gluconate 135, KCl 5, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 5, HEPES 5, Mg-ATP 5; or Cs_2SO_4 110, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 5, HEPES 5, Mg-ATP 5, tetraethylammonium (TEA)-Cl 5 (pH = 7.2). The former and latter solutions were used to observe spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs, respectively), respectively. The patch-pipettes had a resistance of 10–20 MΩ. The sEPSCs were recorded at a holding potential (V_H) of −70 mV where no sIPSCs were observed, since the reversal potential for sIPSCs was near −70 mV. On the other hand, sIPSCs were observed at a V_H of 0 mV where sEPSCs were invisible owing to the reversal potential for sEPSCs to be close to 0 mV. Cs^+ and TEA were added to inhibit K^+ channels