Contribution of α_{2A}-adrenoceptor subtype to effect of dexmedetomidine and xylazine on spinal synaptic transmission of mice

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Abstract

Alpha-2A adrenergic receptor (AR) subtype plays an important role in the analgesic effect of α_2-AR agonists. Here, we examined the effects of α_2-AR agonists, dexmedetomidine and xylazine, on spinal synaptic transmission in newborn C57BL/6J and α_{2A}-AR mutant mice. Spinal reflex potentials, the monosynaptic reflex potential (MSR) and the slow ventral root potential (sVRP), were measured in isolated spinal cords. The compound action potential was measured in isolated lumbar nerve. Dexmedetomidine and xylazine suppressed both the MSR and sVRP in a concentration-dependent manner. In α_{2A}-AR mutant mice, sVRP suppression by dexmedetomidine was greatly weakened, while that by xylazine (30–100 μM) showed only slight attenuation. A high concentration (300 μM) of xylazine completely suppressed the sVRP, even in α_{2A}-AR mutant mice spinal cords, and also suppressed the compound action potential. MSR suppression by these α_2-AR agonists had no difference between wild-type and α_{2A}-AR mutant mice. These results suggest that sVRP suppression by dexmedetomidine and xylazine is mainly mediated by α_{2A}-AR. In addition, a high concentration of xylazine inhibits conduction of the action potential, which is not mediated by α_{2A}-AR. α_2-AR is not responsible for the dexmedetomidine- and xylazine-mediated inhibition of the MSR.

Key words

α-2 adrenoceptor

spinal cord

reflex potential

dexmedetomidine

xylazine

Abbreviations:

ACSF, artificial cerebrospinal fluid; AR, adrenoceptor; MSR, monosynaptic reflex potential; sVRP, slow ventral root potential

1. Introduction

The α_2-adrenoceptor (AR) agonists medetomidine, dexmedetomidine and xylazine are widely used as sedatives or analgesics for animals and humans. The α_2-adrenergic system is important for anti-nociception at the spinal cord level (Pertovaara, 2006; 2013), which is associated with the effect of noradrenaline released from descending inhibitory neurons from the brain (Millan, 2002). The α_2-ARs are classified into α_{2A}, α_{2B} and α_{2C} subtypes (Bylund et al., 1994), and α_{2A}- and α_{2C}-ARs are expressed in the spinal cord (Stone et al., 1998). α_{2A}-AR have presynaptic inhibitory effect on transmitter release and this effect is related to analgesia (Pertovaara, 2013).

Functional knockout α_{2A}-AR mice developed by hit-and-run gene targeting express a point mutation (D79N) in α_{2A}-AR gene (MacMillan et al., 1996). Dexmedetomidine inhibits spontaneous locomotor activity and nociceptive response in wild-type (WT) mice, but not in D79N-homozygous (D79N-homo) mice in behavior experiments, indicating the involvement of α_{2A}-ARs in the effects of dexmedetomidine (Hunter et al., 1997). A study examining the intrathecal application of dexmedetomidine to D79N-homo mice suggests its analgesic effects at spinal level via α_{2A}-ARs (Stone et al., 1997); however, a high concentration of dexmedetomidine also caused analgesia in D79N-homo mice. Dexmedetomidine may activate another subtype of adrenoceptors,

such as α_{2C}-ARs (Taiji et al., 2004), and/or imidazoline receptors (Wikberg et al., 1991). Alternatively, high dose of dexmedetomidine may activate α_{2A}-ARs harboring a point mutation because they retain the ability to stimulate signal transduction pathways (MacMillan et al., 1996), but its precise mechanisms are still unknown.

The isolated spinal cords from newborn rats are useful for investigating the spinal action of analgesics *in vitro* (Otsuka and Konishi, 1974). In this model, electrical stimulation of the lumbar dorsal root evokes changes in two potentials in the corresponding ipsilateral ventral root: the monosynaptic reflex potential (MSR) (reaching a peak within milliseconds) and the slow ventral root potential (sVRP) (lasting about 20–30 s). The MSR and sVRP reflect reflex motor activity and spinal nociceptive transmissions, respectively, in the rat. The sVRP results from activation of primary afferent C fibers (Akagi et al., 1985; Faber et al., 1997) and is sensitive to morphine (Yanagisawa et al., 1985) and dexmedetomidine (Otsuguro et al., 2005).

If the spinal cord is prepared from newborn mice, it may be maintained in an artificial physiological solution without damage to the spinal neurons because the mouse spinal cord is smaller than that of the rat. Therefore, it is possible to quantitatively examine the involvement of receptors or channels in spinal motor and nociceptive reflex pathways such as MSR and sVRP in knockout mice lacking the target

receptors or channels.

Here, we first characterized the reflex responses in spinal cords isolated from newborn mice and compared them with those from newborn rats. Second, we examined the contribution of α_{2A}-ARs to the dexmedetomidine-evoked inhibition of MSR and sVRP in the spinal cords of D79N mice (MacMillan et al., 1996). These mice has been well-characterized in *in vivo* behavior studies (Hunter et al., 1997; Stone, et al., 1997). Third, we examined the effects of xylazine on the MSR and sVRP and compared them with the effects of dexmedetomidine.

2. Materials and methods

2.1. Animals

All animal care and experimental protocols were approved by the institutional animal care and use committee, Graduate School of Veterinary Medicine, Hokkaido University. Every effort was made to minimize animal suffering and to reduce the number of animal used. C57BL/6J mice were purchased from Clea Japan (Tokyo, Japan). Breeding pairs of B6.129S2-*Adra2a*^{tm1Lel}/J mice, which are heterozygous ($\alpha_{2A}^{+/-}$) for a point mutation (D79N) in α_{2A}-ARs (McMillan et al., 1996) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and bred to obtain wild-type

(WT), heterozygous (D79N-hetero) and functional knockout mice (D79N-homo). Mice were genotyped by treatment of restriction enzyme Nhe1 to PCR product, according to the method described in the web database of B6.129S2-*Adra2a*^{tm1Lel}/J mice in The Jackson Laboratory (http://jaxmice.jax.org/strain/002777.html). Wistar rats were purchased from Clea Japan (Tokyo, Japan). Mice and rats of both sexes (1–6 days old) were used in the study.

2.2. Electrophysiological measurement

Spinal reflex potentials were recorded in mice using methods established in rats (Otsuguro et al., 2006, 2011; Kawamoto et al., 2012) with some modifications. Briefly, newborn mice were killed by decapitation. The spinal cords were isolated along with the lumbar dorsal and ventral roots, and then the isolated tissues were hemisected. The hemisected spinal cords were placed in a chamber (volume, 1.5 ml) and superfused with artificial cerebrospinal fluid (ACSF; 138 mM NaCl; 21 mM $NaHCO_3$; 0.6 mM $NaHPO_4$; 3.5 mM KCl; 1.25 mM $CaCl_2$; 1.5 mM $MgCl_2$ and 10 mM glucose) at a flow rate of approximately 2.5 ml/min. The temperature of the bath was maintained at 27 ± 2°C. The ACSF was gassed with 95% O_2 and 5% CO_2 (pH 7.3). Suction electrodes for stimulation and recording were placed on the dorsal root (L2–L4) and the ipsilateral

ventral root, respectively. The dorsal root was stimulated every 2 min by a single square wave pulse (40V, 200 μs), and the magnitudes of the MSR and sVRP were expressed as the peak amplitude (mV) and the depolarization integrals (mV·s) over the resting potential of the ventral root, respectively (Fig. 1A). The preparations were allowed to equilibrate for at least 1 h prior to recording.

The compound action potential was recorded from the central cut end of the L4 lumbar nerve. The L4 lumbar nerve was isolated together with the L4 dorsal root, which was cut near the spinal cord. When the lumbar nerve was electrically stimulated (40 V, 200 μs), the compound action potential consisted of two waves, each of which is reported to be mediated by A and C primary afferent fibres in the rat (Faber et al., 1997). The magnitude of the compound action potential was expressed as the peak amplitude (mV).

The effects of drugs were evaluated according to the mean value of three responses recorded approximately 30 min after drug applications, and were expressed as a percentage of three mean responses measured before drug applications. Drugs were dissolved in ACSF and applied to the spinal cord. Cumulative application were performed to compose the concentration-response curve. Antagonists were applied 30 min after the application of agonists.

Electrical responses were detected with a high gain amplifier (MEZ-8300, Nihon Kohden, Tokyo, Japan). The MSR was recorded by a thermal arraycorder (WR7900, Graphtec, Yokohama, Japan) with a sampling time of 40 μs. The sVRP and compound action potential were digitized by an analog/digital converter (PowerLab, AD Instruments, Cattle Hill, Australia) with a sampling time of 25 ms and 100 μs, respectively. The sVRP and compound action potential data were stored in a personal computer and analyzed with Chart 5 (AD Instruments) software.

2.3. Data analysis and statistical procedures

Results were expressed as the mean ± S.E.M. Statistical comparisons between two groups were performed using an paired Student's *t*-test, and between three groups using Dunnett's test (Excel 2007). *P*-values of less than 0.05 were considered significant.

2.4. Materials

Dexmedetomidine hydrochloride, JP1302 dihydrochloride, efaroxan hydrochloride and idazoxan hydrochloride were purchased from Tocris (Bristol, UK). Atipamezole hydrochloride and xylazine hydrochloride were purchased from Sigma (St.

Louis, MO).

3. Results

3.1. Reflex potentials recorded in the newborn mouse spinal cord

Electrical stimulation (40 V, 200 μs) at the dorsal root evoked two types of responses at the corresponding ventral root of the newborn mice spinal cord (Fig. 1A). The amplitude of the first peak response (n=6) was 9.3 ± 0.5 mV with a time-to-peak of 4.7 ± 0.3 ms, which was followed by a very slow and long-lasting response (a peak amplitude of 1.5 ± 0.2 mV) with a time-to-peak of 1.5 ± 0.5 s. In the newborn rat spinal cord, the peak amplitude of the MSR was 11.1 ± 0.7 mV with a time-to-peak of 6.2 ± 0.2 ms, which was followed by the sVRP (a peak amplitude of 1.3 ± 0.1 mV) with a time-to-peak of 2.7 ± 0.2 s (n=6). The response of the first and second peaks in mice showed similar time course to the MSR and sVRP in the rat. Therefore we referred to first and second responses in mouse isolated spinal cord as the MSR and sVRP.

Next, we examined the effect of morphine on reflex potentials in the mouse spinal cord (Fig. 1B and C). The reflex potentials were evoked every 2 min and morphine (3 nM–1 μM) was applied to the spinal cord for 30 min. Bath application of morphine suppressed sVRP in a concentration-dependent manner. Naloxone (0.3 μM),

an opioid receptor antagonist, recovered the sVRP inhibited by morphine. As shown in Fig. 1B, in most experiments, the peak amplitude of the MSR increased with time. Time-dependent increase of MSR is also shown in isolated rat spinal cord (Iwasaki et al., 2013; Kawamoto et al., 2012; Otsuguro et al., 2011). Morphine did not affect the MSR. Similar results have been reported in newborn rat spinal cords (Otsuguro et al., 2005).

3.2. Effects of α_2-AR agonists on reflex potentials in the mouse spinal cord

The effects of dexmedetomidine and xylazine on spinal reflex potentials evoked by electrical stimulation were examined in the spinal cords of newborn mice. Bath application of dexmedetomidine (1–300 nM) resulted in the concentration-dependent inhibition of the MSR and sVRP (Fig. 2A). The sVRP was more sensitive to dexmedetomidine than the MSR. Atipamezole (10 μM), a non-selective α_2-AR antagonist, recovered the inhibition of the sVRP (78 ± 8% of control, n=4), but not that of the MSR, in response to dexmedetomidine. Xylazine (1–300 μM) also suppressed the MSR and sVRP in a concentration-dependent manner. Atipamezole (10 μM) had no effect on either MSR or sVRP suppression evoked by xylazine (Fig. 2B). Dexmedetomidine was almost 1000 times more potent than xylazine, and both dexmedetomidine and xylazine inhibited the sVRP more effectively than the

MSR (Fig. 2C).

3.3. Effects of α_2-AR agonists on reflex potentials in α_{2A}-AR mutant mouse spinal cord

The effects of α_2-AR agonists on the MSR and sVRP were also examined using spinal cords isolated from mice harboring a point mutation in the α_{2A}-ARs gene. In all three genotypes (WT, D79N-hetero, and D79N-homo types), dexmedetomidine suppressed the MSR in a concentration-dependent manner (Fig. 3A and B). There was no difference between the concentration-inhibition curve for the sVRP in the presence of dexmedetomidine in WT and D79N-hetero mice: however, sVRP inhibition by dexmedetomidine was markedly attenuated in D79N-homo mice (Fig. 3C and D). Even high concentrations of dexmedetomidine (> 1 μM) reduced the sVRP by less than 50%.

Xylazine suppressed the MSR in all three genotypes in a concentration-dependent manner. On the other hand, inhibition of the sVRP in D79N-homo mice in the presence of xylazine (30 and100 μM) was significantly weaker than that in the other two genotypes. A high concentration of xylazine (300 μM) completely abolished the sVRP in all three genotypes.

3.4. Effects of antagonists on α_2-AR agonist-evoked suppression of reflex potentials

As dexmedetomidine was effective at inhibiting the sVRP in D79N-homo mice, we further examined the underlying mechanisms. It is possible that receptors other than α_{2A}-AR are involved in the effects of dexmedetomidine, or that α_{2A}-AR harboring a point mutation is activated by higher concentrations of dexmedetomidine. To examine these possibilities, we evaluated the effects of both α_2-AR subtype specific antagonists and imidazoline receptor antagonists.

In D79N-homo mice, treatment with dexmedetomidine (1 μM) for 30 min inhibited the sVRP, which was recovered by atipamezole (10 μM) but not by JP1302 (10 μM), an α_{2C}-AR subtype specific antagonist (Fig. 5A and B). Efaroxan (10 μM) and idazoxan (10 μM), imidazoline receptor I_1 and I_2 antagonists, respectively, tended to attenuate dexmedetomidine-induced inhibition of the sVRP, but the results were not significantly different (Fig. 5C). On the other hand, dexmedetomidine (1 μM)-induced inhibition of the MSR was not reversed by atipamezole, JP1302, efaroxan, or idazoxan.

Treatment with xylazine (30 μM) for 30 min inhibited the sVRP in C57BL/6J mice (25 ± 4% of control, n=4) (Fig. 6A) without affecting the MSR; however, it had little effect on the sVRP in D79N-homo mice (83 ± 3 % of control, n=7) (Fig. 6B). Atipamezole (10 μM) greatly attenuated xylazine-induced inhibition of the sVRP in WT

mice (88 ± 2 % of control, n=4), but had no effect on that in D79N-homo mice (78 ± 4% of control, n=7) (Fig. 6C), suggesting the involvement of α_{2A}-AR in the inhibition of sVRP induced by xylazine at this concentration.

To identify the mechanisms underlying xylazine (30 μM)-mediated inhibition of the sVRP, we next examined the effects of JP1302, efaroxan and idazoxan in D79N-homo mice. None of these agents affected sVRP inhibition evoked by xylazine (30 μM) (Fig. 6D). Neither did they affect the MSR.

3.5. Effect of α_2-AR agonists on action potential conduction

As shown in Fig. 4D, a high concentration of xylazine abolished the sVRP in all genotypes; thus we next examined the non-specific inhibitory effects of xylazine.

The compound action potential was recorded in the L4 dorsal lumbar nerve isolated from newborn mice. We measured the peak amplitudes of the first and second compound action potential waves (Fig. 7A), which originate from the A and C fibers, respectively, in rats (Faber et al., 1997). In C57BL/6J mice, a high concentration (1 μM) of dexmedetomidine did not affect the first (98 ± 5% of control, n=3) and second waves (108 ± 20% of control, n=3) of the compound action potential (Fig. 7A); however, xylazine (300 μM) reduced the amplitudes of both waves (Fig. 7B), and the amplitude

of the second wave (46 ± 10% of control, n=5) was decreased by more than that of the first (79 ± 4% of control, n=5) (Fig. 7C), suggesting that the nerve fibers with slower conduction velocity are sensitive to xylazine. Similar results were obtained using D79N-homo mice (Fig. 7D).

4. Discussion

Here, we developed a new method of examining reflex potentials in spinal cords isolated from newborn mice. Spinal cords isolated and hemisected from newborn rats can be well maintained in an artificial physiological solution because newborn spinal neurons survive for a long time under the hypoxic conditions *in vitro*. In the present experiments, we measured two types of reflex potential in the newborn mouse spinal cord; these potentials resembled the MSR (reflecting motor reflex activity) and the sVRP (associated with nociceptive pathways) in rats. In addition, the slow reflex potentials in the mouse were inhibited by morphine in a concentration-dependent manner, similar to the rat (Otsuguro et al., 2005), indicating that the reflex potentials recorded in mouse spinal cord corresponded to the MSR and sVRP in the rat spinal cord.

Adrenergic system is important for analgesia in the CNS (Pertovaara, 2013,

Gonçalves et al., 2015). A functional knockout α_{2A}-ARs mouse, which harbors a point mutation (D79N-homo), was developed by hit-and-run gene targeting (MacMillan et al., 1996). Behavioral experiments showed that dexmedetomidine, a non-selective α_2-AR agonist, inhibited nociceptive responses in these mice. On the other hand, the analgesic effects of high concentrations of dexmedetomidine in D79N-homo mice are inconsistent, i.e., Hunter et al. (1997) showed complete dissipation, while Stone et al. (1997) showed retention. Therefore, we further examined the effect of dexmedetomidine on the reflex potentials in D79N-homo mice. We found that dexmedetomidine inhibited the sVRP in normal mice; however, this was reversed by atipamezole, a non-selective α_2-AR antagonist. Although dexmedetomidine-evoked inhibition of the sVRP in D79N-homo mice was greatly attenuated, dexmedetomidine inhibited the sVRP when used at higher concentrations.

This inhibitory response to dexmedetomidine observed in D79N-homo mice was abolished by atipamezole. Atipamezole inhibits imidazoline receptors in addition to α_2-AR (Sjöholm et al., 1992). Therefore, other α_2-AR subtypes or imidazoline receptors might be associated with the inhibitory effect of dexmedetomidine. In D79N-homo mice, however, sVRP inhibition by dexmedetomidine was not affected by JP1302 (an α_{2C}-AR specific antagonist), efaroxan (an imidazoline receptor I_1 antagonist), or idazoxan (an

imidazoline receptor I_2 antagonist). Taken together, these results suggest that the inhibitory effect of dexmedetomidine on the sVRP is due to specific inhibition of α_{2A}-ARs, and that higher concentrations of dexmedetomidine are capable of activating α_{2A}-ARs harboring a point mutation, which retain the ability to activate signal transduction pathways in the mouse. This conclusion is supported by the fact that D79N-homo mice show an 80% reduction in functional α_{2A}-AR binding (MacMillan et al., 1996).

Like dexmedetomidine, xylazine inhibited the sVRP in a concentration-dependent manner, although xylazine was 1000-times less potent than dexmedetomidine. The marked difference in the sensitivity of α_2-ARs in the mouse spinal cord to these drugs seems similar to that in the rat (Otsuguro et al., 2005). Xylazine (30 μM)-induced inhibition of the sVRP in WT mice was abolished by atipamezole and was greatly attenuated in D79N-homo mice, suggesting that α_{2A}-ARs play an important role in sVRP suppression in response to xylazine at this concentration. α_2-AR would be responsible receptor for this effect. By contrast, inhibition of the sVRP in response to high concentrations of xylazine was not greatly affected, even in D79N-homo mice. Mechanisms other than α_{2A}-ARs seem to be involved in sVRP inhibition in response to high concentrations of xylazine. A high concentration of

xylazine (300 μM) completely suppressed the sVRP in all genotypes. Compound action potentials, especially the second slow component, were greatly suppressed by xylazine in WT and D79N-homo mice, suggesting the inhibition of action potential conduction due to the stabilizing effect of the drug on the cell membrane.

Hunter et al., (1997) reported that a dexmedetomidine-induced reduction in spontaneous locomotor activity was greatly attenuated in D79N-homo mice. In the present study, however, there was no difference in dexmedetomidine-induced inhibition of the MSR in WT and D79N-homo mice. Moreover, dexmedetomidine-induced MSR inhibition was not affected by α_2-AR and imidazoline receptor antagonists, suggesting no involvement of α_2-ARs and imidazoline receptors. Similar results were obtained for xylazine-induced inhibition of the MSR. It seems likely that spontaneous locomotor activity is independent of reflex motor activity in the mouse. Further experiments are required to identify the mechanisms underlying MSR suppression by dexmedetomidine.

5. Conclusion

Inhibition of the sVRP via α_{2A}-ARs in response to dexmedetomidine and xylazine is suggested to be due to activation of α_{2A}-ARs in the mouse spinal cord, thereby generating the anti-nociceptive effect of these drugs. Dexmedetomidine and

xylazine also attenuated the MSR via unknown mechanisms, which require further investigation. The mouse isolated spinal cord model seems to be useful for examining spinal motor and nociceptive reflex pathways, in genetically modified mice.

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Figure legends

Fig. 1. Effects of morphine on reflex potentials in spinal cords isolated from newborn mice. (A) Representative traces of reflex potentials evoked by electrical stimulation (arrowhead). The magnitudes of the monosynaptic reflex potential (MSR) and the slow ventral root potential (sVRP) were measured as the peak amplitude (mV) and the integral of depolarization (mV s) over the resting potential, respectively. (B) The effects of cumulative application of morphine on the MSR and sVRP. Naloxone (0.3 μM) was applied in the presence of morphine (1 μM). Upper traces represent MSRs and sVRPs, in the presence of morphine (1 μM) alone, morphine + naloxone (0.3 μM), or neither (control). The lower panel shows the time course of changes in the MSR and sVRP evoked every 2 min in the presence of morphine (concentrations are indicated in rectangular boxes) and then after the addition of naloxone (0.3 μM). (C) Concentration-response curves for the MSR and sVRP in the presence of morphine. Data are expressed as the mean ± S.E.M. (n=5).

Fig. 2. Effects of dexmedetomidine (DEX) and xylazine (XYL) on reflex potentials in the spinal cord of newborn C57BL/6J mice. DEX (A) and XYL (B) were cumulatively

applied and atipamezole (ATI, 10 μM) was applied in the presence of the highest concentration of α_2-AR agonists. Upper and lower traces (A and B) indicate the representative monosynaptic reflex potentials (MSRs) and slow ventral root potentials (sVRPs), and the lower panel indicates the time course of suppression of spinal reflex potentials evoked every 2 min in the presence of DEX (A) or XYL (B) (concentrations are indicated in the rectangular boxes). (C) Concentration-response curves for the MSR and sVRP in the presence of DEX and XYL. Data are expressed as the mean ± S.E.M. (n=4).

Fig. 3. Effect of dexmedetomidine (DEX) on reflex potentials in the spinal cord of α_{2A}-AR mutant (D79N-hetero and D79N-homo) and wild-type (WT) mice. Representative traces of the monosynaptic reflex potentials (MSR) (A) and slow ventral root potential (sVRP) (C) from three genotypes in the presence and absence of DEX (300 nM). Concentration-response curves for the MSR (B) and sVRP (D) in the three genotypes in the presence of DEX. Data are expressed as the mean ± S.E.M. (n=4–5) *P < 0.05 WT (Dunnett's test).

Fig. 4. Effect of xylazine (XYL) on reflex potentials in the spinal cord of α_{2A}-AR

mutant (D79N-hetero and D79N-homo) and wild-type (WT) mice. Representative traces of the monosynaptic reflex potentials (MSR) (A) and slow ventral root potential (sVRP) (C) in the three genotypes in the presence of XYL (30 and 300 μM) and in its absence (control). Concentration-response curves for the MSR (B) and sVRP (D) in the three genotypes in the presence of XYL. Data are expressed as the mean ± S.E.M. (n=5–6) **P* < 0.05 *vs.* WT (Dunnett's test).

Fig. 5. Effects of α_{2A}-AR and imidazoline receptor antagonists on dexmedetomidine (DEX)-induced inhibition of the monosynaptic reflex potentials (MSR) and slow ventral root potential (sVRP) in D79N-homo mice. Thirty minutes after DEX-induced MSR and sVRP suppressions, atipamezole (ATI, 10 μM) (A) or JP1302 (10 μM) (B) was added. (C) Summary of the effects of ATI, JP1302, efaroxan (EFA, 10 μM) and idazoxan (IDA, 10 μM) on DEX (1 μM)-evoked suppression of the MSR and sVRP. Control (ctl) indicates responses in the presence of DEX before application of the antagonist. Responses in the presence of the antagonists shown beneath the columns were obtained 30 min after application. Data are expressed as the mean ± S.E.M. (n=5–6) **P* < 0.05 (paired Student's *t*-test).

Fig. 6. Effects of α_2-AR and imidazoline receptor antagonists on xylazine (XYL)-induced inhibition of the slow ventral root potential (sVRP) in C57BL/6J and D79N-homo mice. Atipamezole (ATI, 10 μM) was applied to spinal cords from C57BL/6J (A) and D79N-homo (B) mice 30 minutes after sVRP suppression induced by XYL (30 μM). (C) Summary of the effects of ATI (10 μM) on XYL (30 μM)-evoked suppression of the sVRP in C57BL/6J mice. Control (ctl) indicates responses in the presence of XYL before ATI was applied. (D) Summary of the effects of ATI, JP1302 (10 μM), efaroxan (EFA, 10 μM) and idazoxan (IDA, 10 μM) on XYL (30 μM)-evoked suppression of the sVRP in D79N-homo mice. Control (ctl) indicates responses in the presence of XYL before antagonist application. The response in the presence of antagonists shown under the columns were obtained 30 min after application. Data are expressed as the mean ± S.E.M. (n=4–6) *$P < 0.05$ (paired Student's *t*-test).

Fig. 7. Effects of dexmedetomidine (DEX, 1 μM) and xylazine (XYL, 300 μM) on the compound action potential recorded in the mouse dorsal root. The compound action potential was evoked every 2 min by a single electrical stimulation (arrowheads). Representative responses (first and second waves) before (control) and after application of DEX (A) and XYL (B) in C57BL/6J mice. Summary of the effects of DEX and XYL

on the compound action potential in C57BL/6J (C) and D79N-homo mice (D). Data are expressed as the mean ± S.E.M. (n=3–5) *$P < 0.05$ (paired Student's *t*-test).

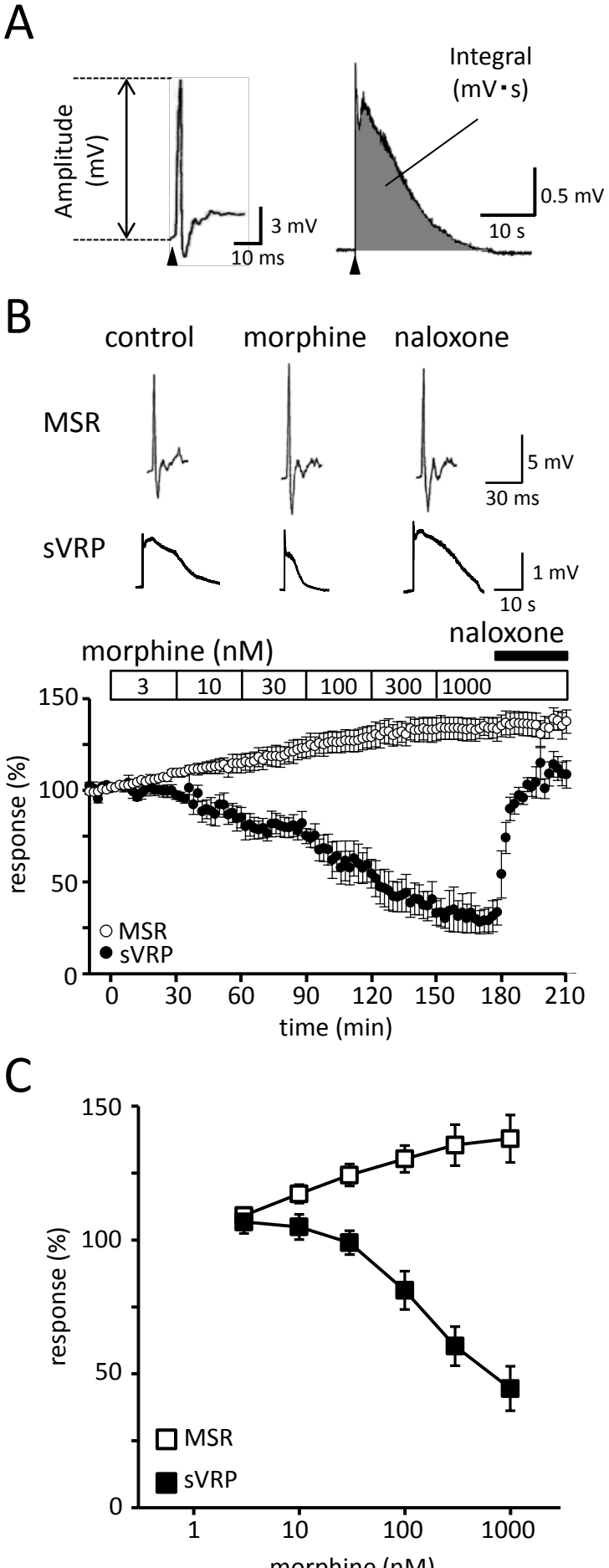
A
Amplitude (mV)
3 mV
10 ms
Integral (mV・s)
0.5 mV
10 s
B
control
morphine
naloxone
MSR
5 mV
30 ms
sVRP
1 mV
10 s
morphine (nM)
naloxone
3
10
30
100
300
1000
150
100
50
0
response (%)
MSR
sVRP
0
30
60
90
120
150
180
210
time (min)
C
150
100
50
0
response (%)
MSR
sVRP
1
10
100
1000
morphine (nM)

Fig. 1

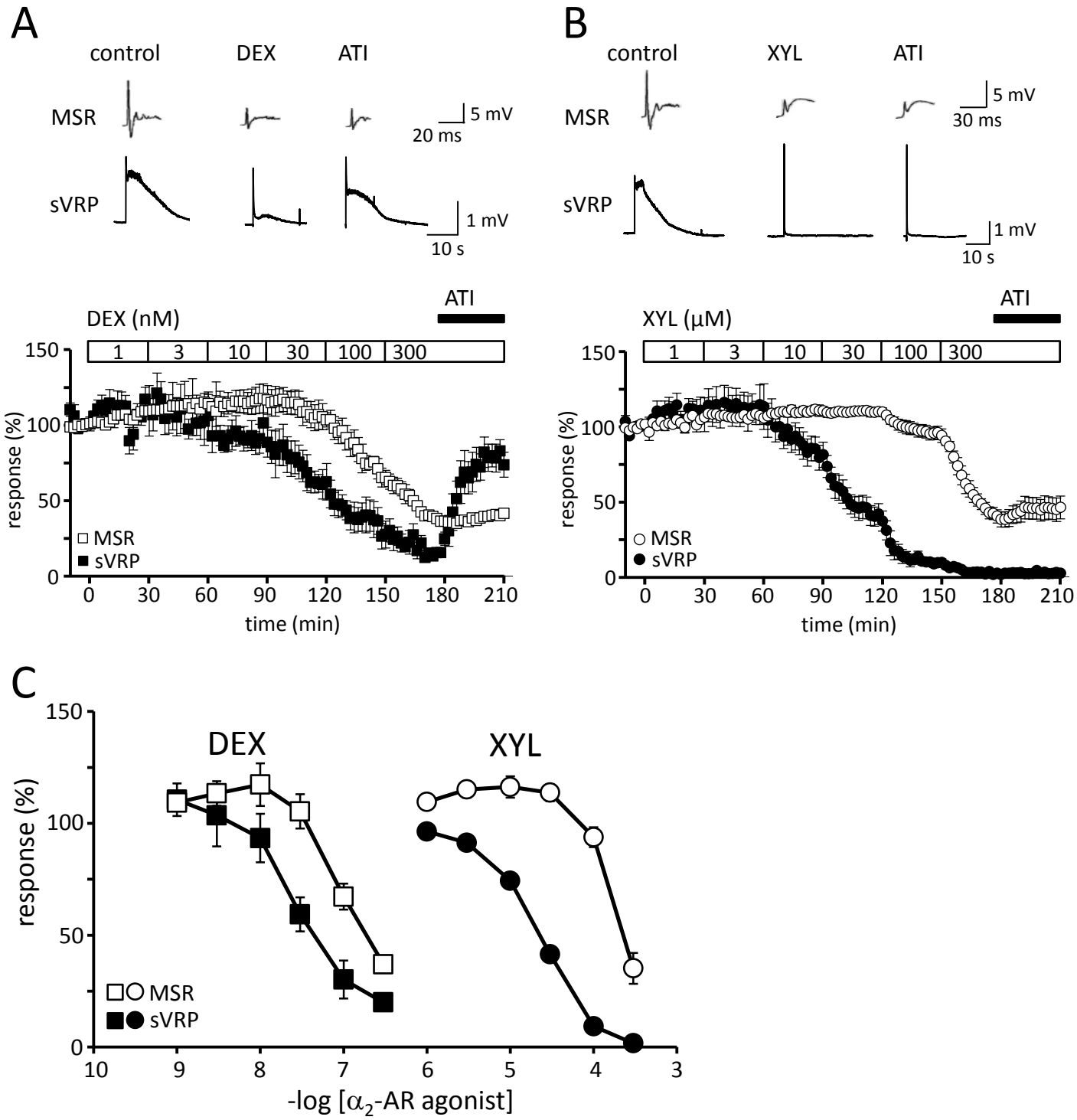
A
control
DEX
ATI
MSR
sVRP
5 mV
20 ms
1 mV
10 s
B
control
XYL
ATI
MSR
sVRP
5 mV
30 ms
1 mV
10 s
DEX (nM)
ATI
1
3
10
30
100
300
response (%)
MSR
sVRP
time (min)
XYL (µM)
ATI
1
3
10
30
100
300
response (%)
MSR
sVRP
time (min)
C
DEX
XYL
response (%)
MSR
sVRP
-log [α2-AR agonist]

Fig. 2

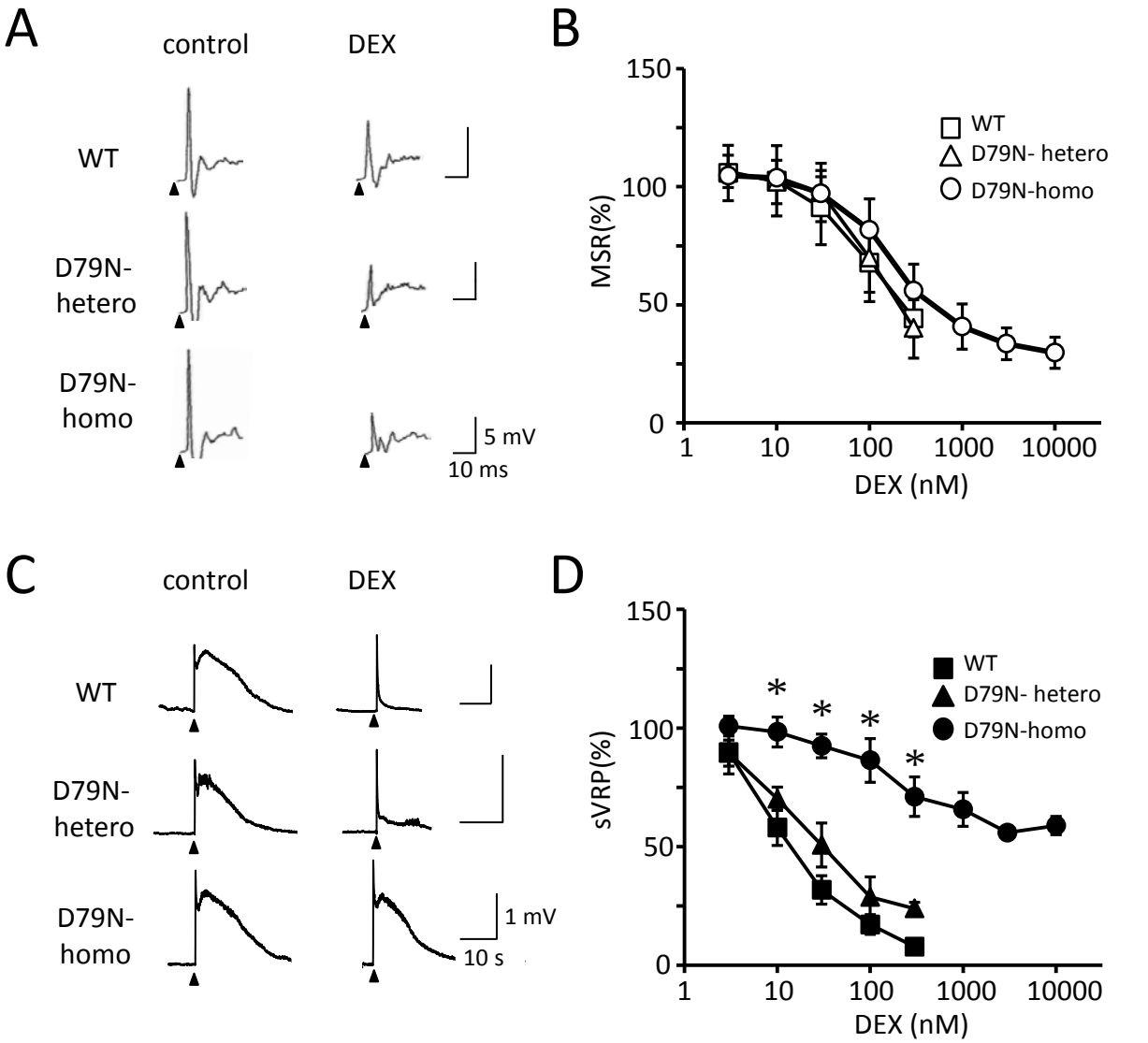
A
control
DEX
WT
D79N-
hetero
D79N-
homo
5 mV
10 ms
B
150
100
50
0
MSR(%)
WT
D79N- hetero
D79N-homo
1
10
100
1000
10000
DEX (nM)
C
control
DEX
WT
D79N-
hetero
D79N-
homo
1 mV
10 s
D
150
100
50
0
sVRP(%)
*
*
*
*
WT
D79N- hetero
D79N-homo
1
10
100
1000
10000
DEX (nM)

Fig. 3

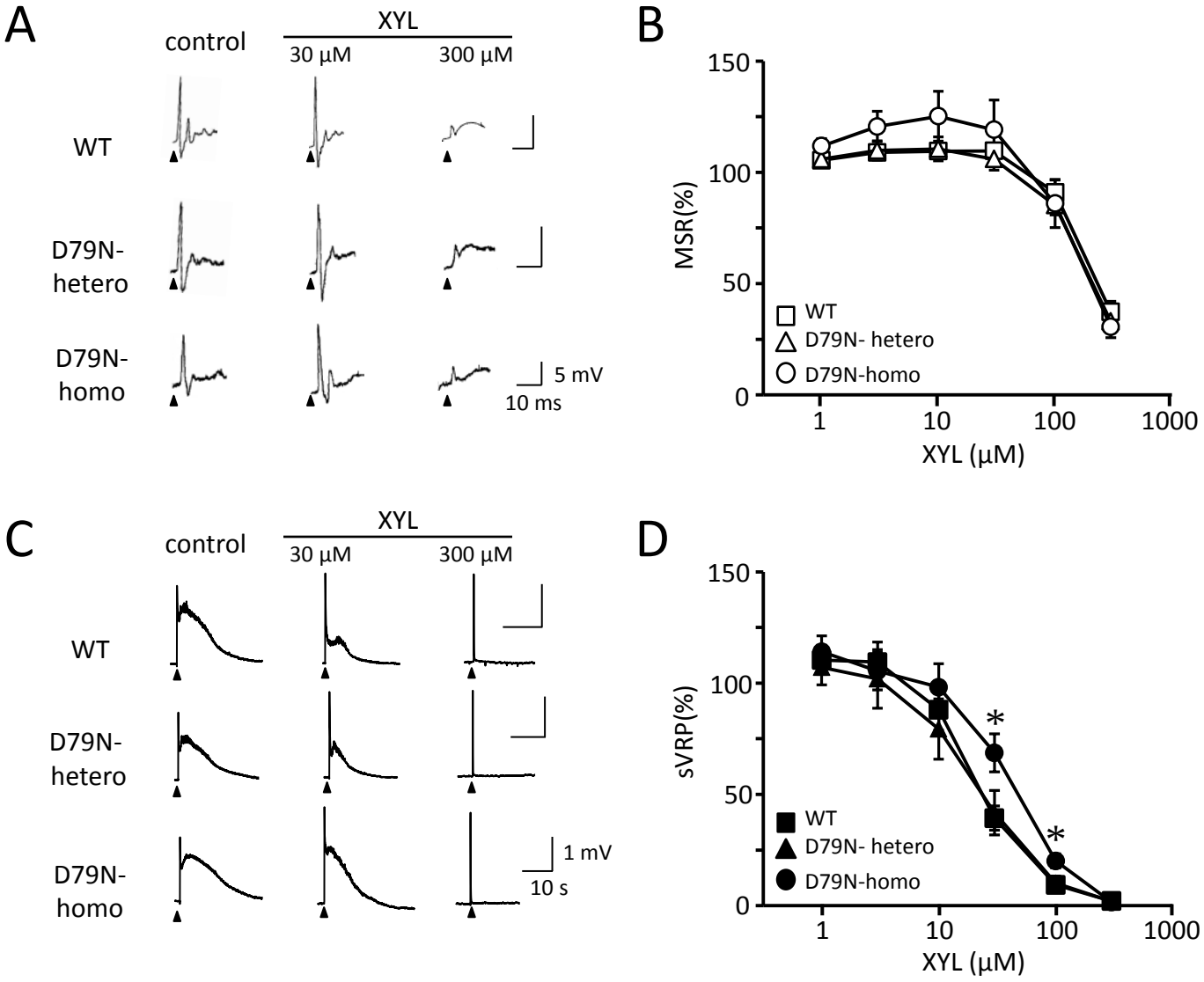
A
control
XYL
30 μM
300 μM
WT
D79N-hetero
D79N-homo
5 mV
10 ms
B
MSR(%)
150
100
50
0
WT
D79N- hetero
D79N-homo
1
10
100
1000
XYL (μM)
C
control
XYL
30 μM
300 μM
WT
D79N-hetero
D79N-homo
1 mV
10 s
D
sVRP(%)
150
100
50
0
WT
D79N- hetero
D79N-homo
*
*
1
10
100
1000
XYL (μM)

Fig. 4

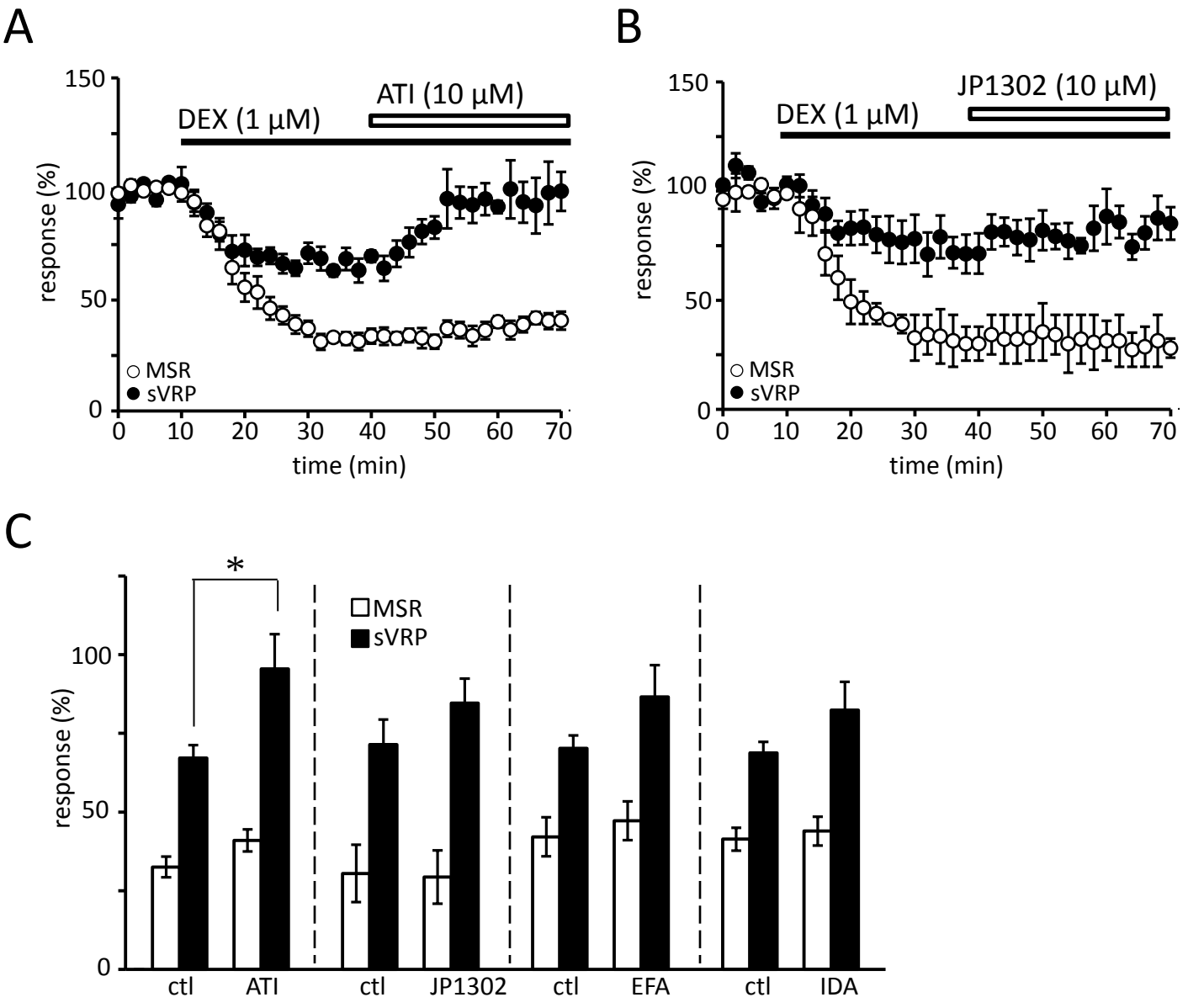
A
DEX (1 μM)
ATI (10 μM)
response (%)
time (min)
MSR
sVRP
B
DEX (1 μM)
JP1302 (10 μM)
response (%)
time (min)
MSR
sVRP
C
response (%)
MSR
sVRP
*
ctl
ATI
ctl
JP1302
ctl
EFA
ctl
IDA

Fig. 5

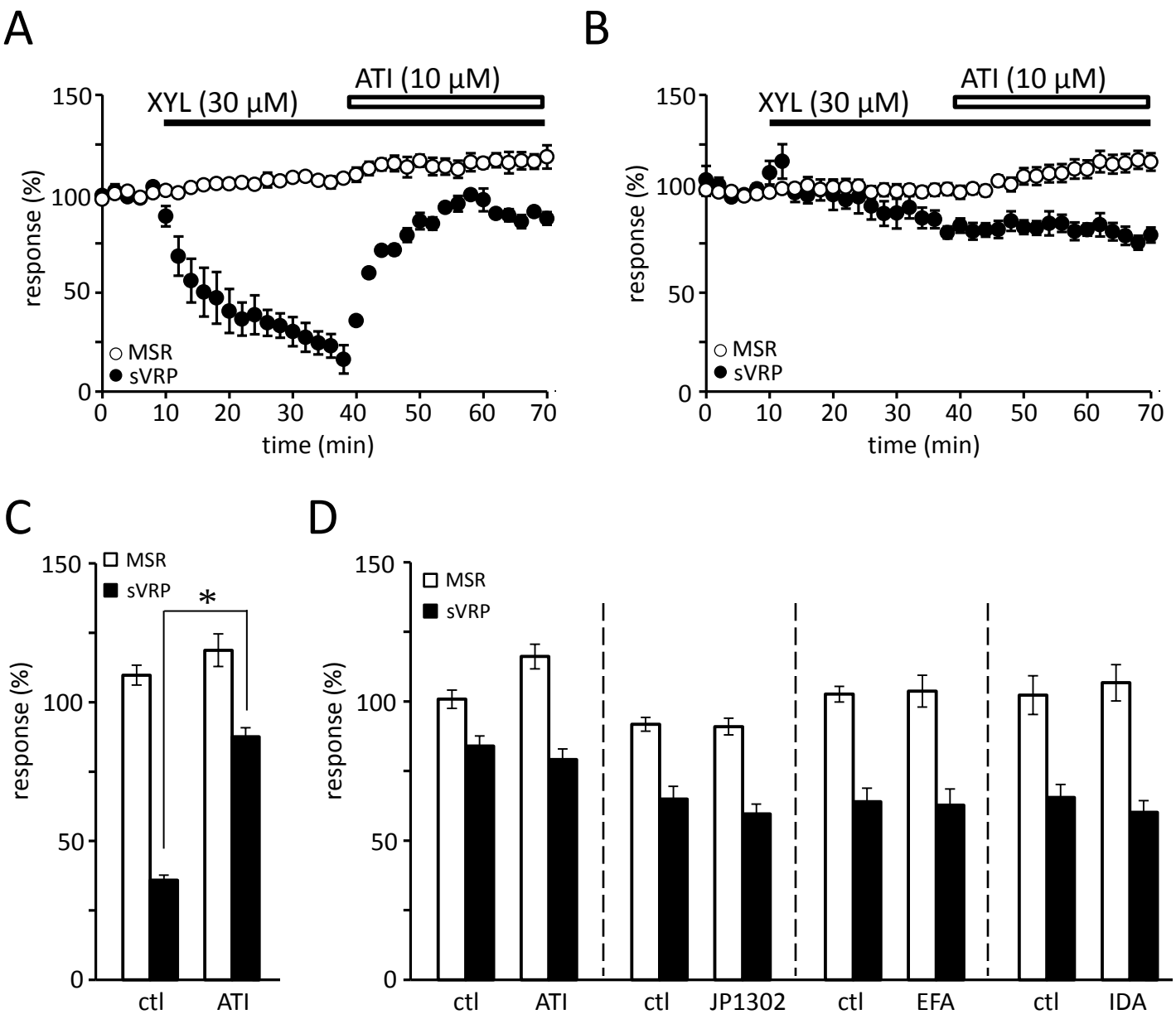
A
B
XYL (30 µM)
ATI (10 µM)
response (%)
time (min)
MSR
sVRP
C
D
*
ctl
ATI
JP1302
EFA
IDA

Fig. 6

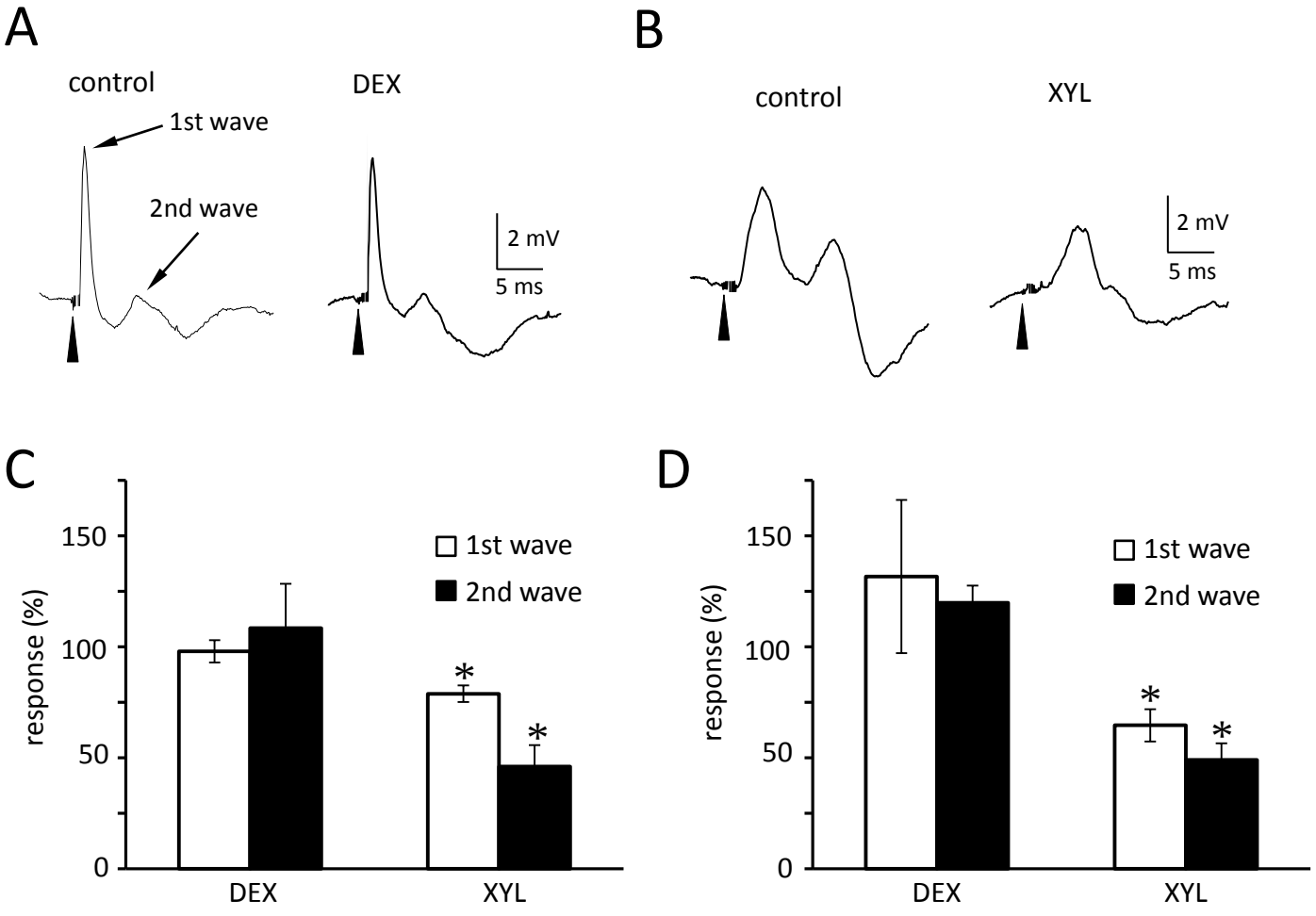
A
control
DEX
1st wave
2nd wave
2 mV
5 ms
B
control
XYL
2 mV
5 ms
C
response (%)
150
100
50
0
1st wave
2nd wave
*
*
DEX
XYL
D
response (%)
150
100
50
0
1st wave
2nd wave
*
*
DEX
XYL

Fig. 7